RESEARCH REPORT

1.Introduction.

I am still focussing on the development of rapid techniques to detect point mutations in mammalian cells. There are at least two categories of point mutations; (1) those for which you already know the DNA sequence change, and (2) those that might be somewhere in the gene that you are interested in, but you are not sure where. Point mutations that are in the first category include the human hemoglobinopathies (sickle cell anemia, etc), alpha-1-antitrypsin deficiency and HLA alleles. Point mutations in the second class include the newly arising point mutations responsible for some of the Lesch-Nyhan cases in our collection and (probably) some of the mutations leading to DMD.

Point mutation detection techniques can be divided into a similar two categories; (1) techniques that are only useful if you already know what the mutation is, or (2) Procedures that can 'scan' a large length of DNA or RNA for a single base change. Allele Specific Oligonucleotide (ASO) probing is a procedure for identifying well characterised point mutations. RNase A cleavage, denaturing gradient gel electrophoresis and the strand-displacement assay (Figure One) are all procedures for 'scanning' for new mutations.

Each of these procedures can be used in conjunction with the polymerase chain reaction (PCR) procedure, which can amplify target sequences and simplify the subsequent analyses. PCR has also opened up the possibility of direct DNA sequencing, either to detect a previously known mutation or to look for new ones. In this report I will describe a number of different experimental -point mutation detection systems that I have been exploring. Almost all of these are PCR based. They include denaturing gradient gel electrophoresis and direct sequencing strategies (with Richard Blaszak), and point mutation detection by competitive oligonucleotide priming (with Nikki Nguyen).

2. Optimising PCR Reaction Conditions.

We now have quite a bit of experience with the 'basic' PCR reaction. At first the reaction did not work at all, but fiddling with several variables has identified conditions that give consistently good amplification. Because there are a large number of variables, most results are 'anecdotal'. Below are the final conditions that we arrived at for amplifying fragments of 30

the internal probes. Good signals were not obtained. Sometime was spent determining good conditions for blotting small fragments (Figure Five) and we found that a 1.5% agarose gel, transfered to to 'Zeta-Probe' in o.4N alkali gave a substantially better result than NuSieve and/or genesreen.

Meantime, we also found that primer extension provided an easier and more sensitive assay for the PCR products (Figure Six). We have since avoided the filter hybridisations wherever possible. The identification of DMD deletions by this method is shown in Figure Seven.

4. PCR and Direct DNA Sequencing.

If you can identify a PCR internal primer-extension product on a gel, then you are close to a DNA sequencing strategy. We have tried several times to break down a primer extension product with a dideoxy chain termination ladder, but have not yet done so. An example is shown in Figure eight. There is now published one example of direct DNA sequencing of PCR products from mammalian cells, and we note that they use 36 rounds of PCR followed by a gel purification step. These experiments are ongoing. We emphasise that although we feel that the direct sequencing will be a powerful tool, it will be no means preclude the use of other mutation detection techniques.

5. PCR and Denaturing Gradient Gel Electrophoresis.

The primer extension products mentioned above are suitable for analysis on denaturing gradient gels. We have the gel system running, as shown in Figure 9. So far primer extension products have not yielded products with good melt-transitions (see overhead). We believe this to be due to the short length of the duplexes that we have analysed. We intend to test out longer duplexes.

6. Competetive Oligonucleotide Priming

It is well established that oligonucleotides will bind to complementary DNA even when the homology is not complete. This is the basis of the ASO detection system, and is frequently used in PCR reactions and in site directed mutagenesis strategies. We have found, quite surprisingly, that when two oligonucleotides are supplied to a hybridisation reaction, at low stringency, then a 100% match is strongly favoured over a single base mismatch. We have demonstrated this with several different primers and

C. Flourescent Probes

We are collaborating with Ken Beatty to use the competeive oligo priming system with flourescently labelled oligo's to do simple detection of human disease mutations. Towards this end, I have synthesised oligos with a 5' aliphatic amino group, using commercially available witchcraft, and Ken Beatty has conjugated these to flourescent dyes. The relevant structures are in Figure Twelve. This strategy is being piloted on the *spf* cloned cDNA with flousescein and Texas Red tagged primers.

D. Next

The competetive priming system is ready to try on genomic DNA with flourescent primers. As an added refinement we are trying to construct a solid support for the 'universal' primer, to allow the thing to be automated.

6 Scheme To Revolutionise Mutagen Screening

Finally, I would like to discuss an idea that Grant MacGregor and I have conceived for mutagen screening in mammalian cells. The strategy is shown in Figure Thirteen. The advantages of this scheme are that no selection need be imposed on the mammalian cells and that the precise DNA sequence changes that are induced can be readily obtained. The scheme will take advantage of an on-line ^{32}P detector that can do automated DNA sequencing. This machine is made by a company called EG and G, based in Boston. I have initiated a agreement for them to lend us a machine for two months to try some automated mutation detection strategies. One glitch in the scheme is that the background in the plasmid ligations may be high, but we are optimistic.

7. Summary

We are continuing to develop strategies for point mutation detection. Most are PCR based. PCR plus DNA sequencing and PCR plus denaturing gradient gel electrophoresis are almost working. A new point mutation detection strategy has been developed, based on competition between priming oligonucleotides. Competetive oligonucleotide priming has the potential to replace ASO probing for the detection of previously characterised point mutations.

0349R

RESEARCH REPORT

Introduction

My main aim is to develop improved methods for detection of point mutations in mammalian cells. The techniques being used are ribonuclease A (RNase A) cleavage, denaturing gradient gel electrophoresis, and the polymerase chain reaction (PCR) procedure for the amplification of specific nucleotide sequences. HPRT is serving as a 'model locus' for development of the procedures.

Ultimately we want rapid procedures with the power for the detection of <u>all</u> possible point mutations. Neither RNase A cleavage nor denaturing gradient gel electrophoresis are capable of this in their current form. These procedures also share a problem with Southern and Northern blotting- unique mammalian DNA sequences are rare and difficult to detect. PCR offers an approach to alleviate this limitation.

In this report I will describe a procedure for the detection of point mutations using denaturing gradient gel electrophoresis and PCR, that is under development. I am focusing on this combination of procedures because they offer, for the first time, the potential to detect all point mutations in a simple, rapid, easy manner. Several other strategies for 'molecular diagnostics' using denaturing gradient gels, RNase A cleavage and PCR have been conceived. Some have been explored and will be discussed. The end of the spf mouse study will also be described. Finally, much of this work is dependent on the availability of synthetic oligonucleotides. Our new synthesiser will be up on the 26th of May, and interested people can start placing orders.

Denaturing Gradient Gel Electrophoresis and PCR with polyC Tails.

Denaturing gradient gel electrophoresis can resolve point mutations in double stranded DNA due to the resultant differences in thermal melting temperatures (Tm's) of the duplexes. Because it is the formation of partially denatured molecules which results in changes in electrophoretic mobility, only single DNA base changes which are within the 'melting domains' with the lowest Tm's in DNA duplexes will be detected. Myers et al. have demonstrated that when a duplex is covalently linked to a highly GC rich DNA sequence then the probability of detecting point mutations is increased. This is because the GC 'clamps' have a relatively high Tm, and so all the rest of the duplex must fall into a relatively low Tm region. Myers et al. used the approach of cloning the sequence to be analysed into a vector containing the beta-globin promoter, in order to introduce the GC clamps. This is too cumbersome for routine analysis of mammalian genes. Therefore an alternative approach to introduce the GC clamps will be employed. PolyC tails which are present on the 5' end of oligonucleotides will be introduced into PCR amplified HPRT sequences. The opposing primer for the reaction will be have a ^{32}P end label. After PCR, the sequences of interest will be amplified, labelled and attatched to the GC clamp. These can be loaded directly onto a denaturing gel or alternatively, cut out of a NuSieve agarose gel and then run on the denaturing gel.

Progress towards having this procedure up and running has been directed towards getting the PCR going and trying out the denaturing gradient gels. The PCR works fine on cloned DNA (second figure), using HPRT cDNA primers. PCR of exon 9 HPRT sequences and beta globin DNA sequences have both shown a band visible by ethidium bromide staining after twenty reaction rounds

(third figure). The denaturing gradient gels have been run with 'perpendicular' gradients, and two RNA:RNA hybrids generated from HPRT cDNA cloned into pTZ. Interesting patterns have emerged. The denaturing gradient gels were quite simple to form and run. Currently the limiting factor is oligonucleotides, which have been on order for some time. It is possible that the polyC tailed oligos will pose problems with the synthesis. Once our machine is running I will synthesise a stock of $(C)_n$, and then attempt to ligate this to other oligos using a GGGGNNNN 'splint'.

PCR Diagnosis of DMD

Now that DMD gene sequences are available it is possible to try using some new 'tricks' to speed up DMD diagnosis. Oligos spanning short regions of DMD exons (from within pert 87.4 and 87.25) have been obtained. A high proportion of DMD cases are deleted for these sequences (5 - 20%). In these cases there will be no DNA sequences to prime, therefore no PCR reaction product. HPRT/beta globin/alpha 1 antitrypsin primers can serve as internal controls and amplified products can be identified on agarose gels, or alternatively in a Southern blot or in a series of dot blots with oligos which are internal to the PCR primers.

Sparse Fur OTC

Since Steves last talk, we have conducted a functional analysis of the mutation that we have cloned from the spf mouse. We find that the cloned sequence mimics the previously reported pH dependent change in activity optima that was reported in the original spf mouse. Therefore, we believe that we cloned out the real thing and not some terrible artifact.

Conclusion

RNase A cleavage is no longer the primary focus for mutational analysis. PCR and denaturing gradient gel electrophoresis are currently favored as the best combination of techniques for detecting most point mutations. PolyC tailed oligonucleotide primers are about to be used to directly introduce GC clamps into PCR amplified DNA sequences. The spf mutation seems to have been identified correctly.

1. It is well documented in several families that a new mutation deletion in the DMD locus was recurrent from a mother who had no somatic tissue evidence of the deletion. Presumably these mothers are gametic mosaics for the deletion. The frequency is unknown. I feel all our deletion cases should be reviewed (J.W.) for this setting and revised counseling (P.W.) provided. PND appears indicated until the risk is better documented. Conservative view.

2. The time is ripe to initiate a paper on our deletion cases (J.W.). This data is being put together by A. Roses and I'm sure others. I can help in the case identification 1-200. Beyond this point a review of each report is indicated.

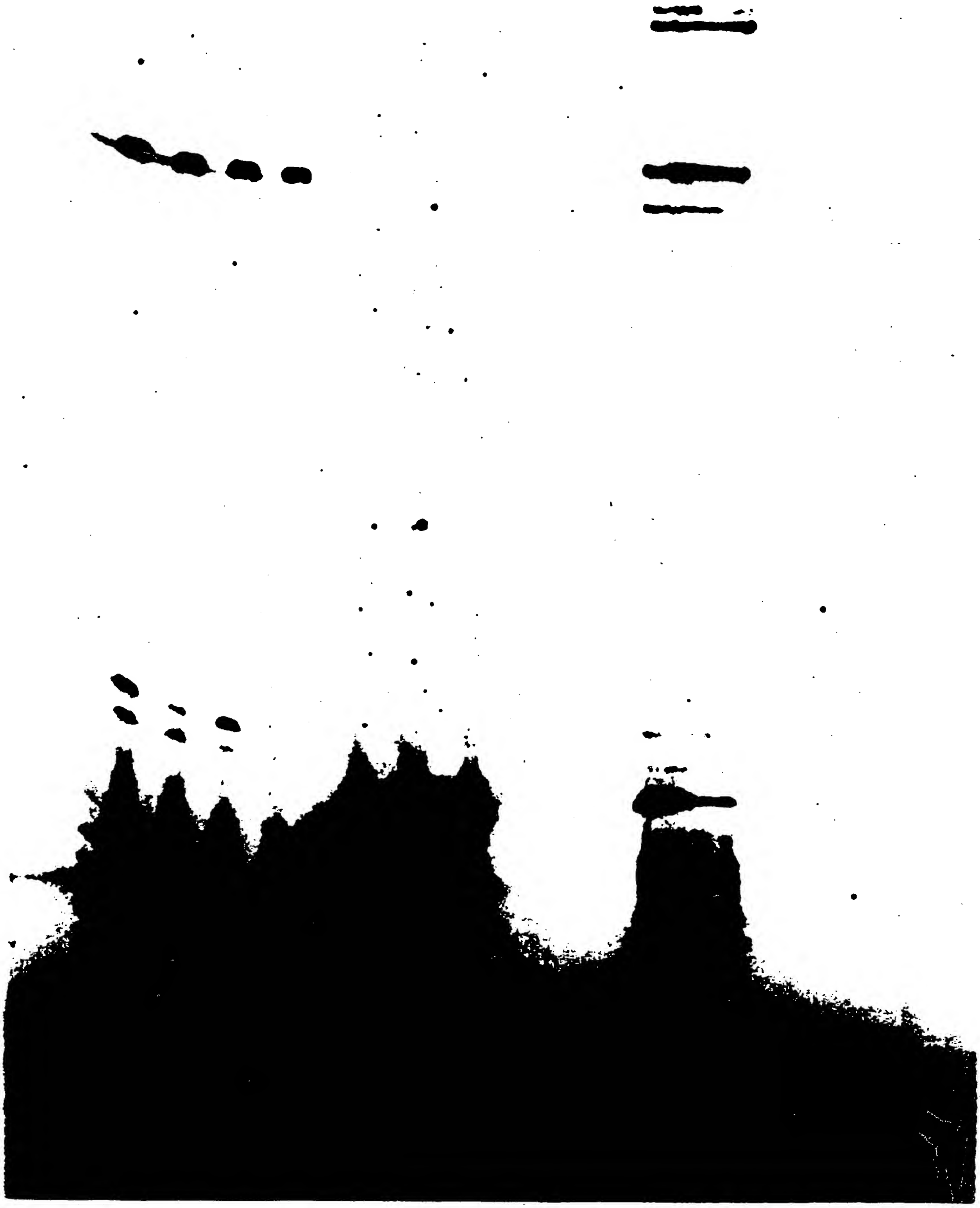
4. I am pleased we can proceed with DMD mouse paper. I suggest a table of RFLPs be added. If we have used Hind III, the gene position of the RFLP can be determined and stated. "Science" is the Target (JC) and submission should follow the L. Kunkel submission to "Cell" - soon. I now think mdx may be Beckers and urge that we examine all independent mdx for exon deletions with our mouse cDNA and consider RNAse A after Northers have been run. Jeff, I think you are logical for this but let's discuss.. To further refine the map position - I will write S. Orkin for CGA probe. We should keep in touch with Ed McCabe on Gkinase. I'd like to use H. Moser's AdLDys probe (T.W.). This may make the present animals more informative. For future animals I feel survey of 100-200 males from MDXC57B/sp with G6PDH/CGP/DMD/G/K/α Galactosidase/ADLD/FACTOR8 is indicated. MDA will accept a grant for this study of MDX/DMD. I need to discuss with Jeff and Tom. Verne Chapman may be sensitive to our doing these studies. We will need to discuss with him.

7. I doubt that L.Kunkel will provide us with materials for simplifying diagnostics. He may try to do it himself. I feel an effort at PCR for the exon used by Jeff to identify our clonses is indicated. The region is small. The flanking sequence is published. We have mutants to test the idea. I'd like to discuss this with Jan and Richard. The long range goal would be a dot/blot for deletions which could span the gene but not examine all exons. The first to establish the principle will lead. MDA will accept a Task Force application on this approach.

C. Th.mas Caskey, M.D.

CTC:htj

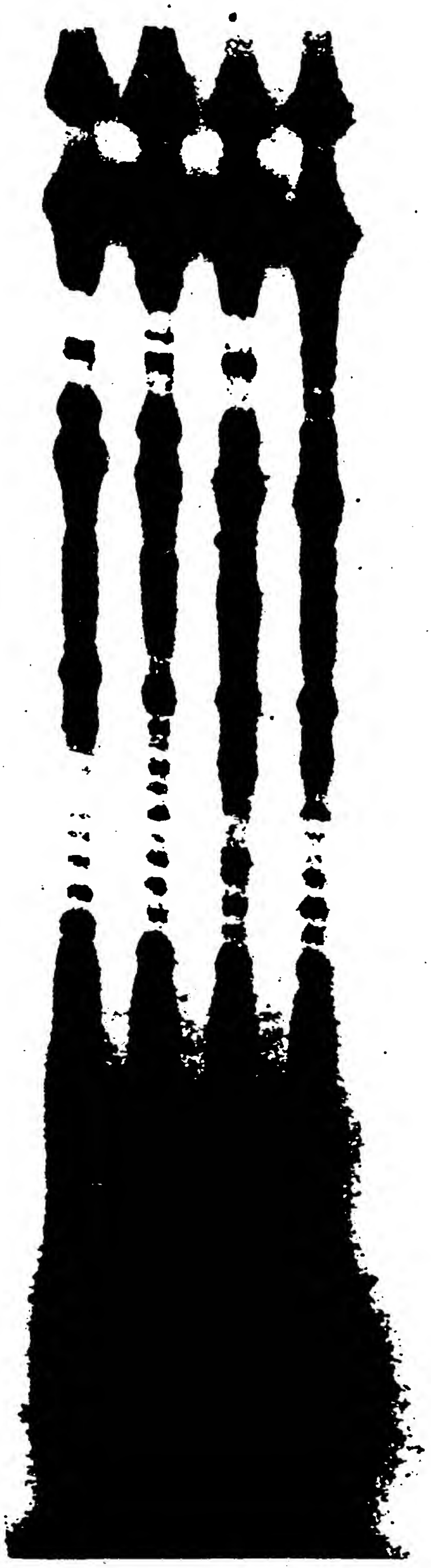
FIGURE SIX



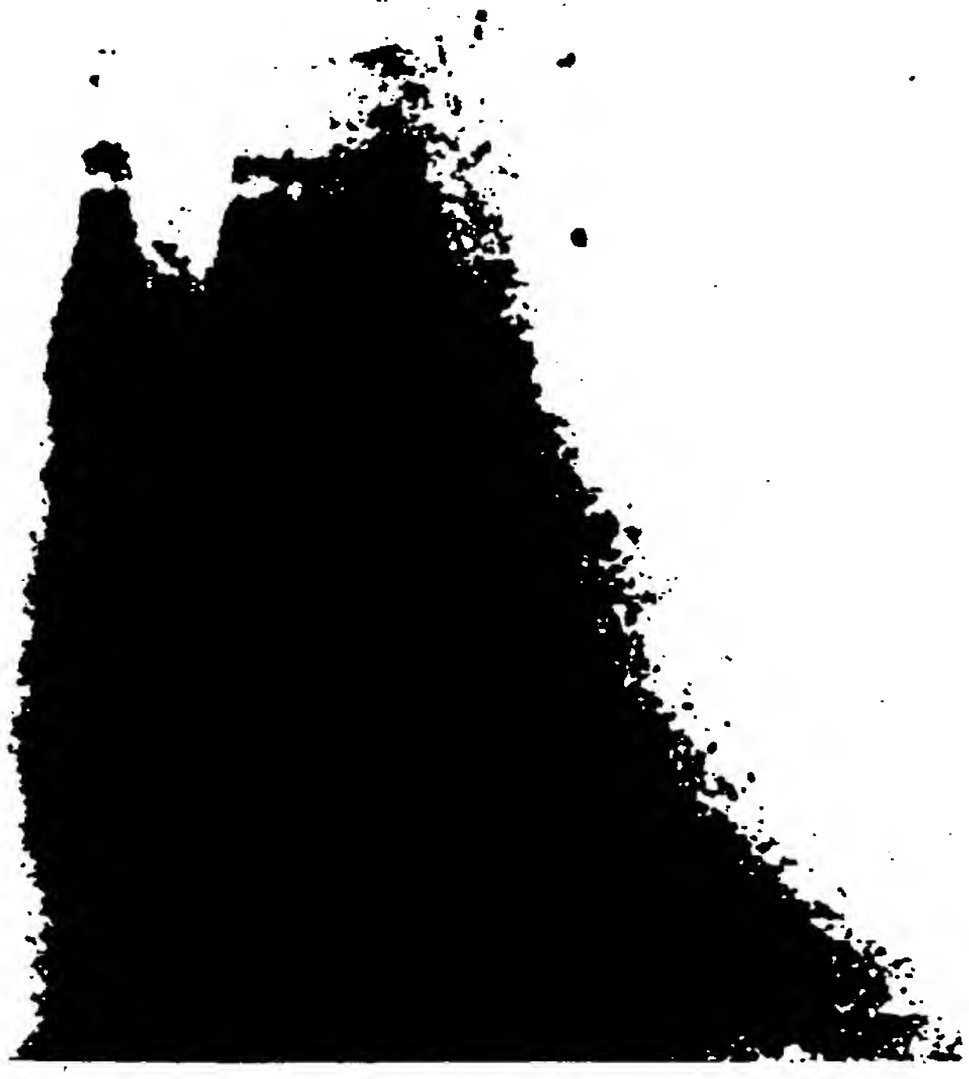
FIGURE

~~EIGHT~~

EIGHT



A T C G

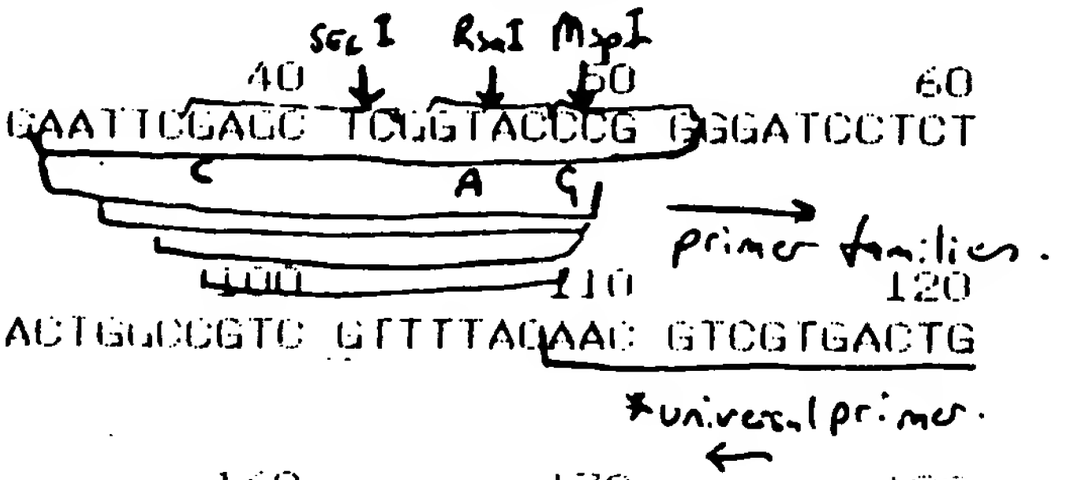


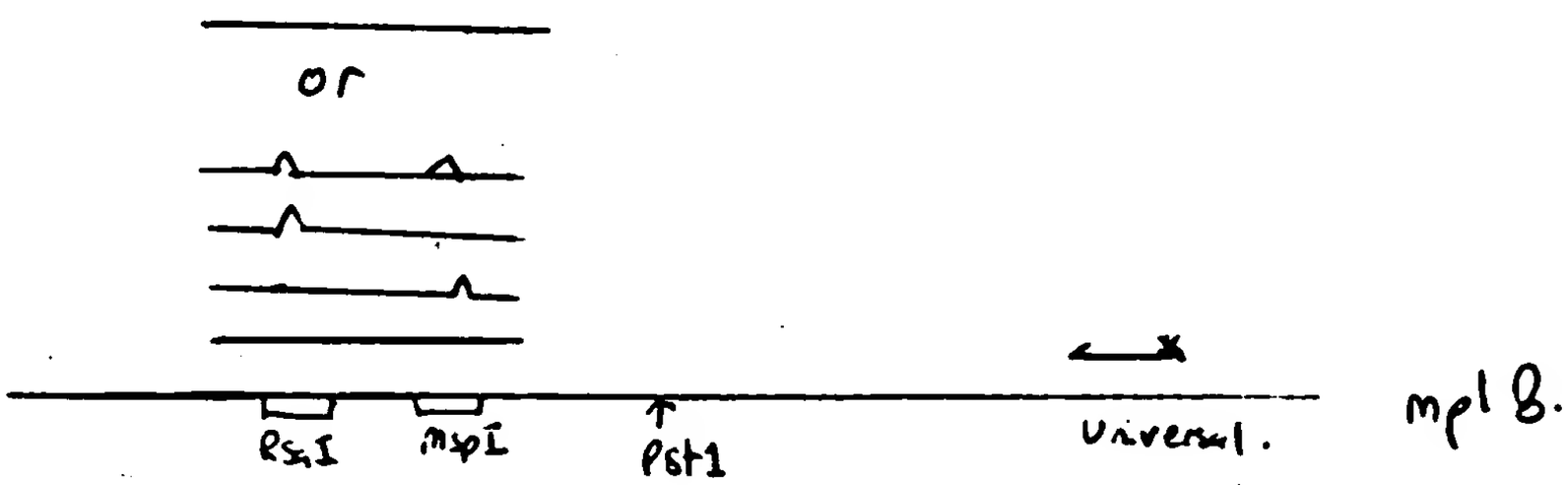
mp18 Current length: 130 ENTRY: Displa

```
        10         20         30         40         50         60
CACACAGGAA ACAGCTATGA CCATGATTAC GAATTCGAGC TCGGTACCCG GGGATCCTCT

        70         80         90        100        110        120
AGAGTCCACC TGCAGGCATG CAAGCTTGGC ACTGGCCGTC GTTTTACAAC GTCGTGACTG

       130        140        150        160        170        180
GGAAAACCCT
```





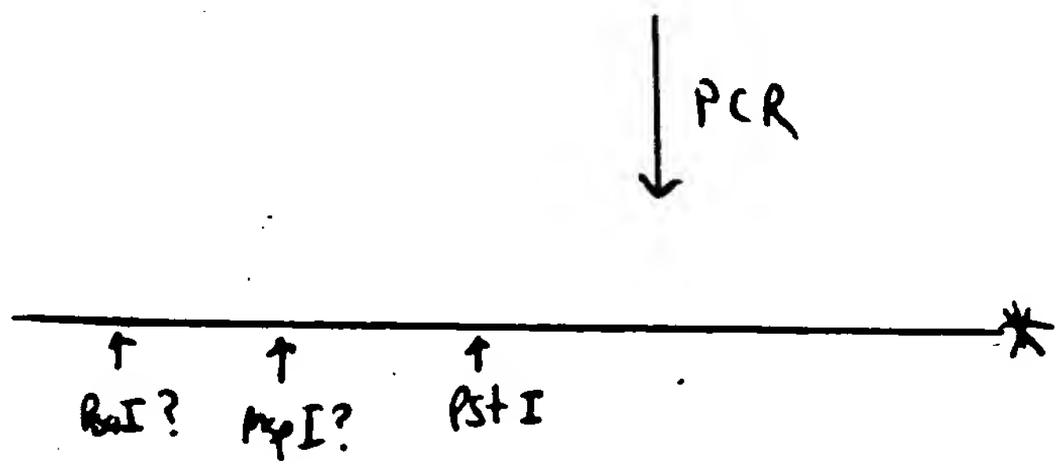
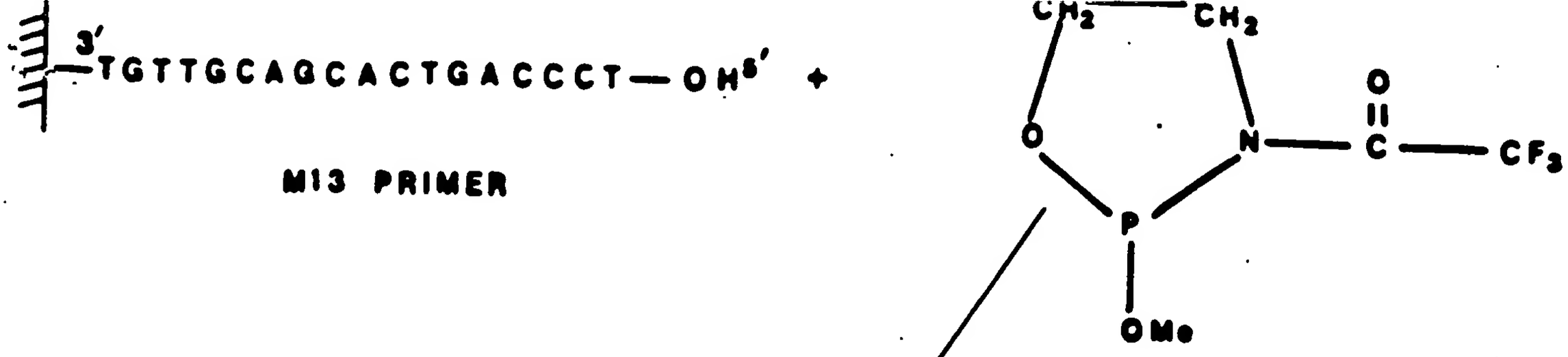


Figure 10.



AMINOLINK—OLIGONUCLEOTIDE PRIMER

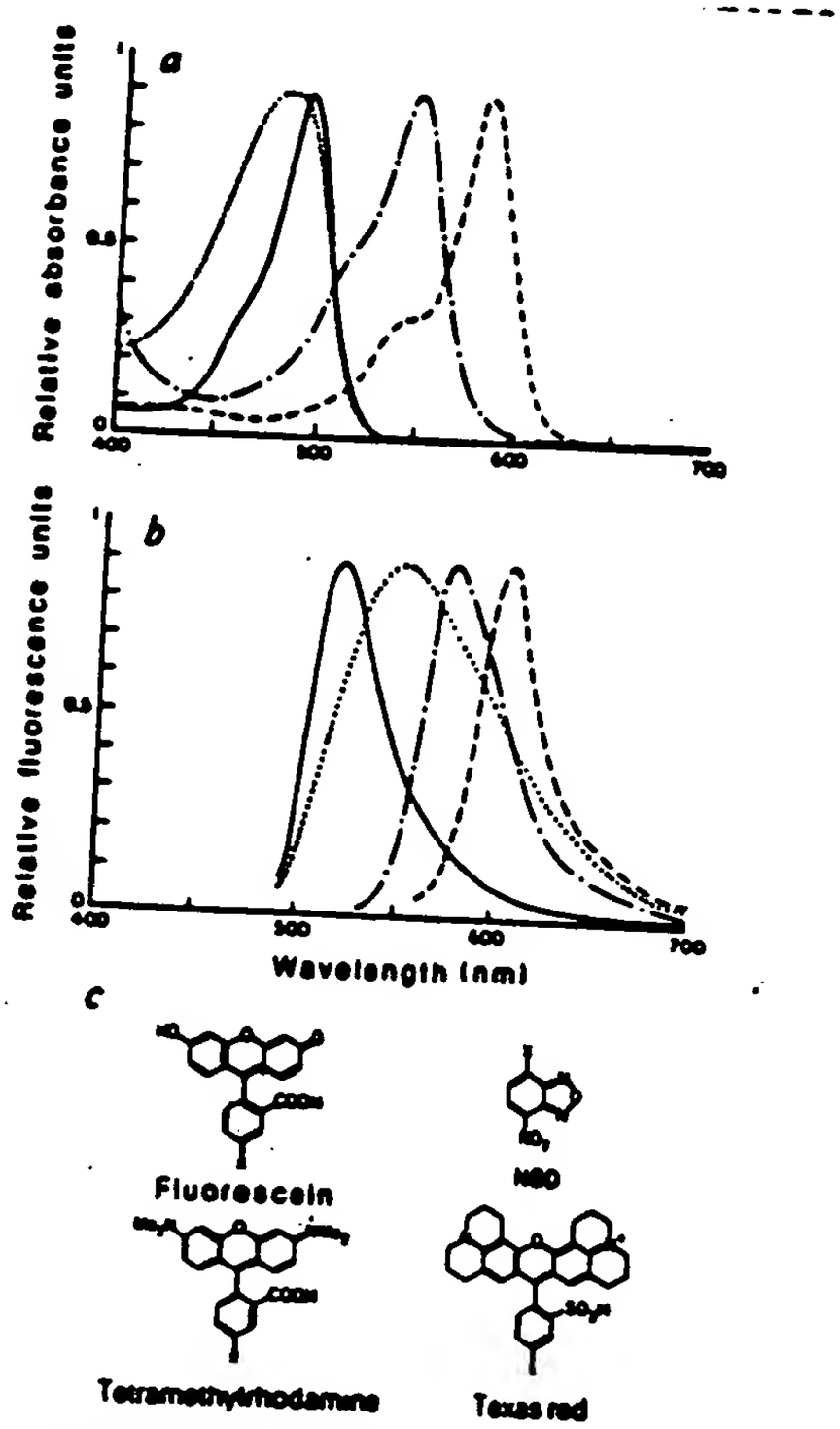
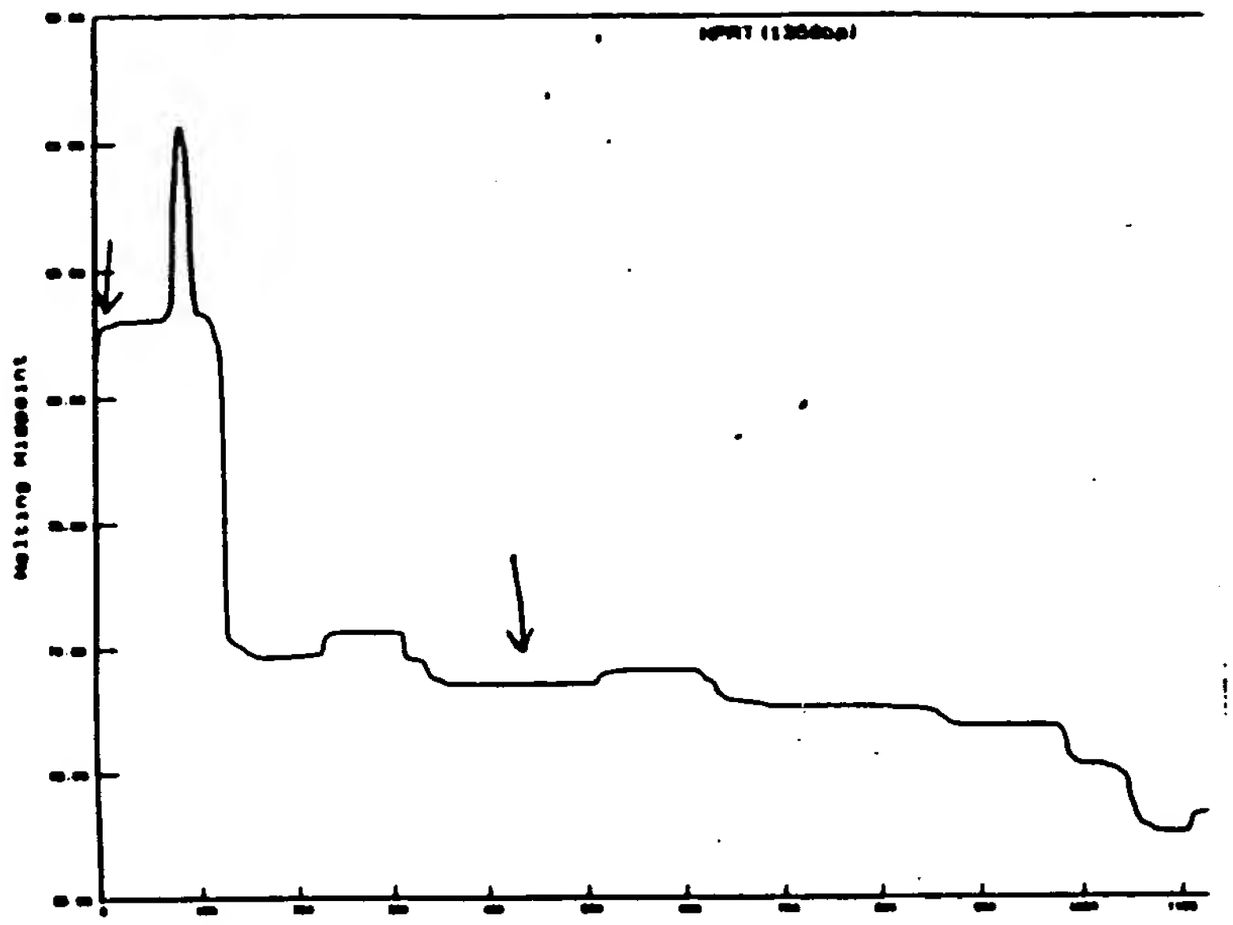
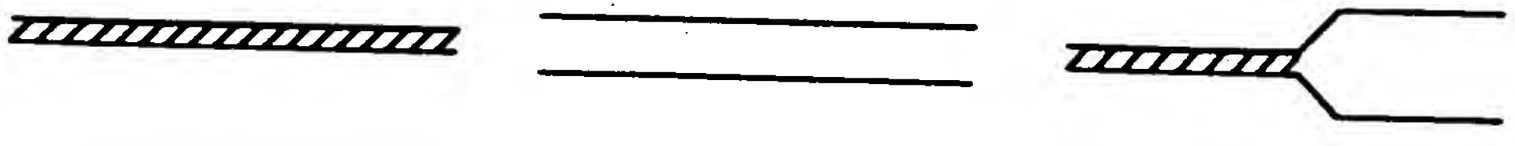
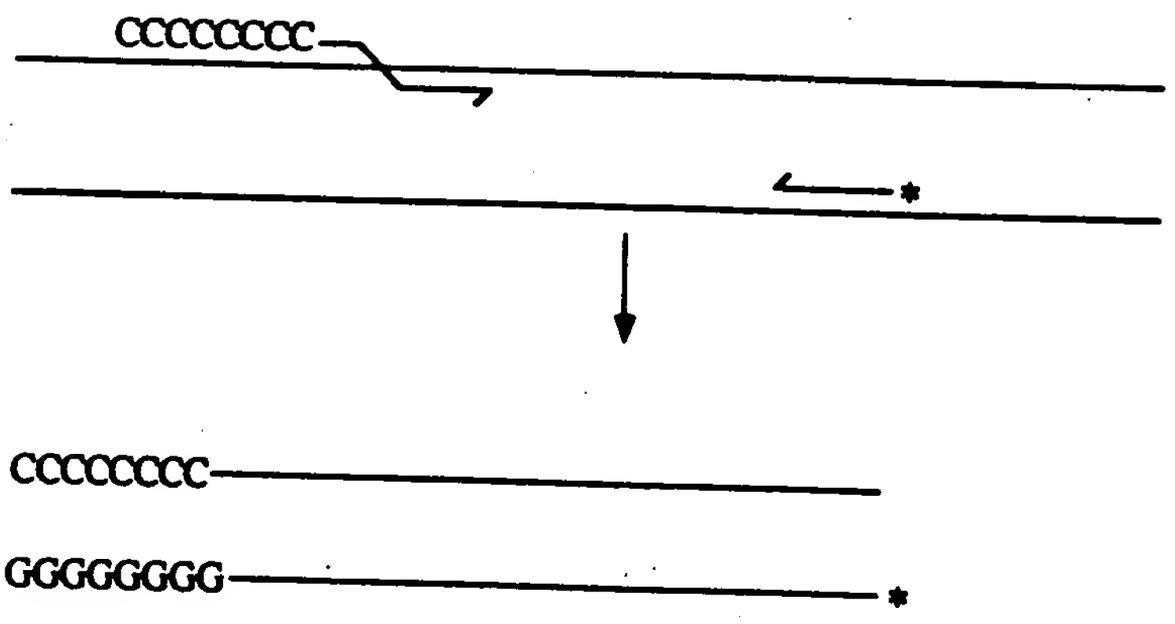
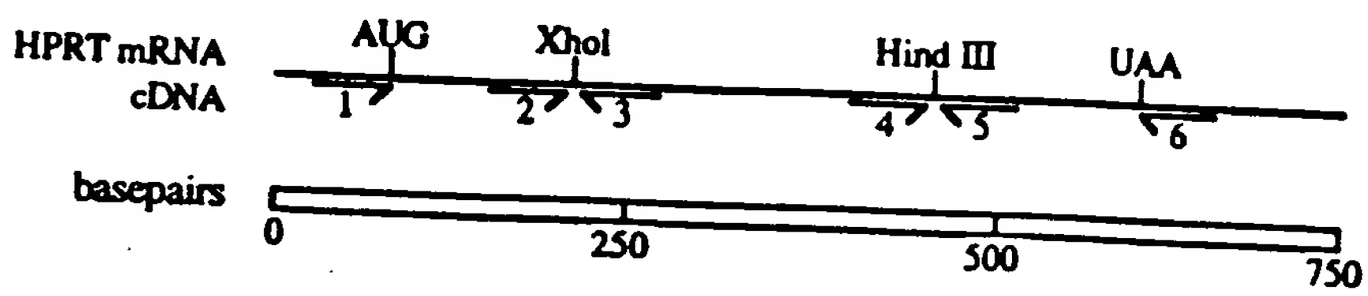


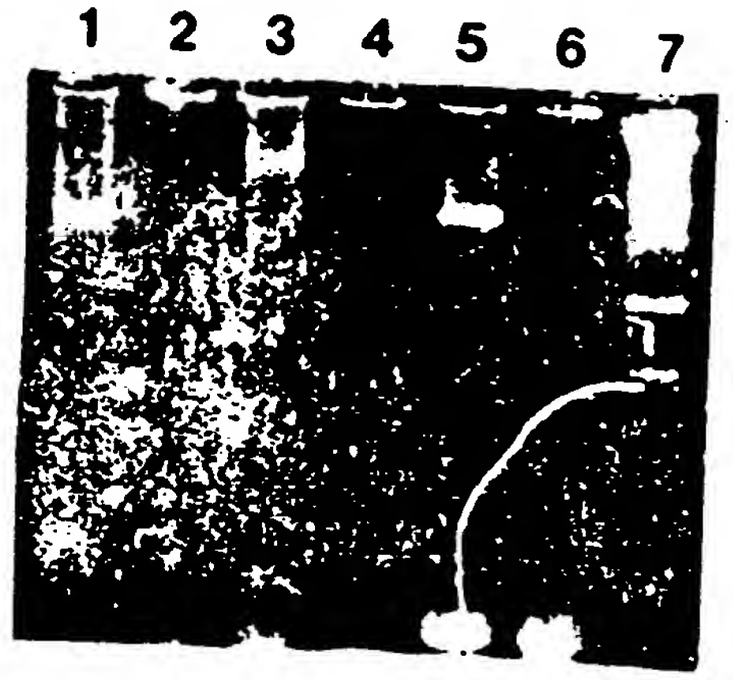
Fig. 2 *a*, Absorption spectra of the four dyes used in the DNA sequenator: ——, fluorescein; ·····, NBD; -·-·-, tetramethylrhodamine; - - - -, Texas Red. *b*, Florescence emission spectra of the four dyes; the same line types as in *a*, are used to denote the dyes. *c*, Chemical structures of the four dyes. X, The moiety to which the dye is bound, for example, an oligonucleotide primer.. **Methods.** All spectra were obtained in 10 mM sodium carbonate buffer, pH 9.0; absorption spectra were taken on an H/P 8451 spectrophotometer; fluorescence spectra were taken on a Perkin-Elmer MPF4 spectrofluorimeter (uncorrected). The following dye derivatives were used for measurements: fluorescein isothiocyanate (FITC), NBD aminohexanoic acid, Texas Red (all from Molecular





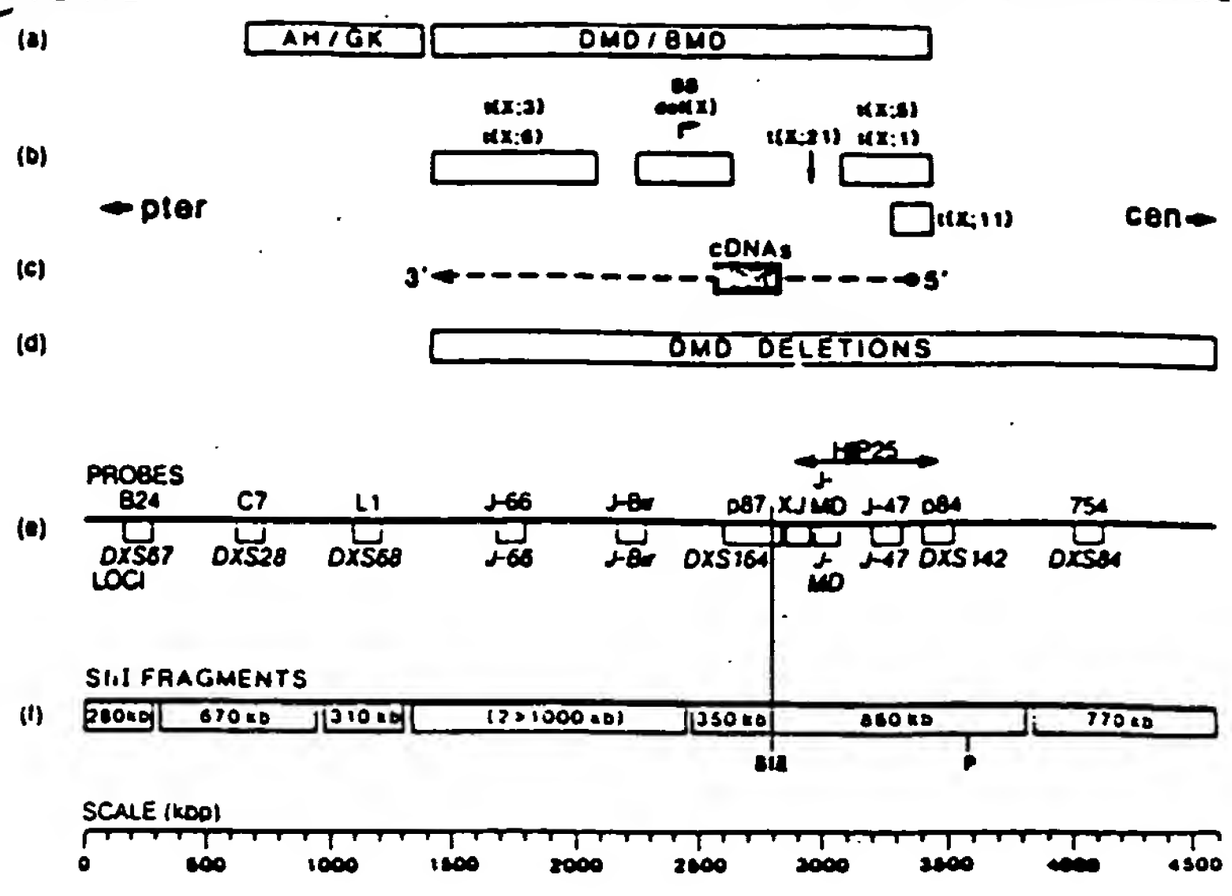

CCCCCCCC
GGGGGGGG


HPRT mRNA
cDNA
AUG
XhoI
Hind III
UAA
1
2
3
4
5
6
basepairs
0
250
500
750


1 2 3 4 5 6 7


20
10
0
HPRT
20
10
0
β-globin
MARKERS

reviews



b

pert 87.4

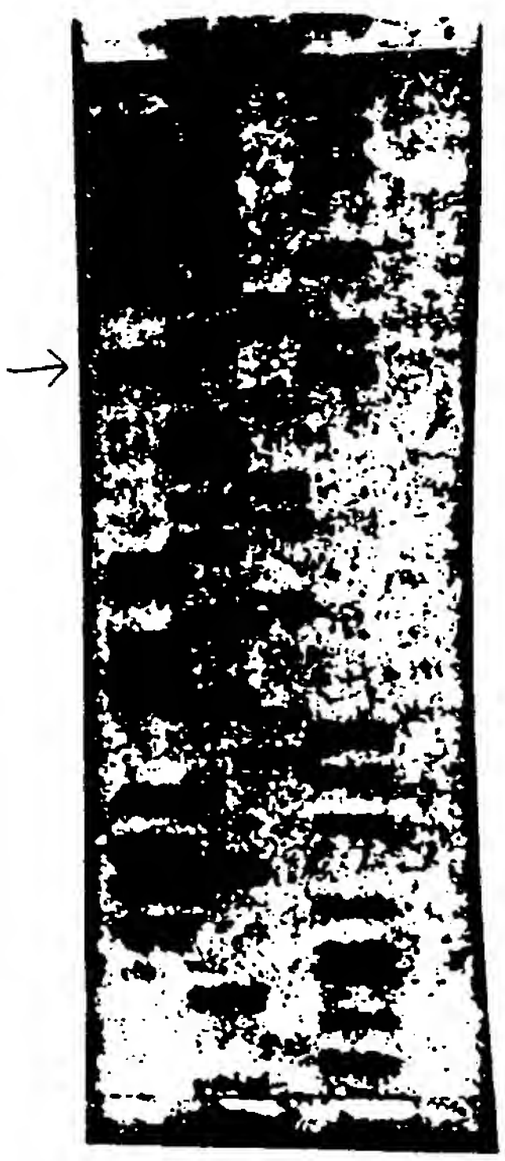
pert 87.25

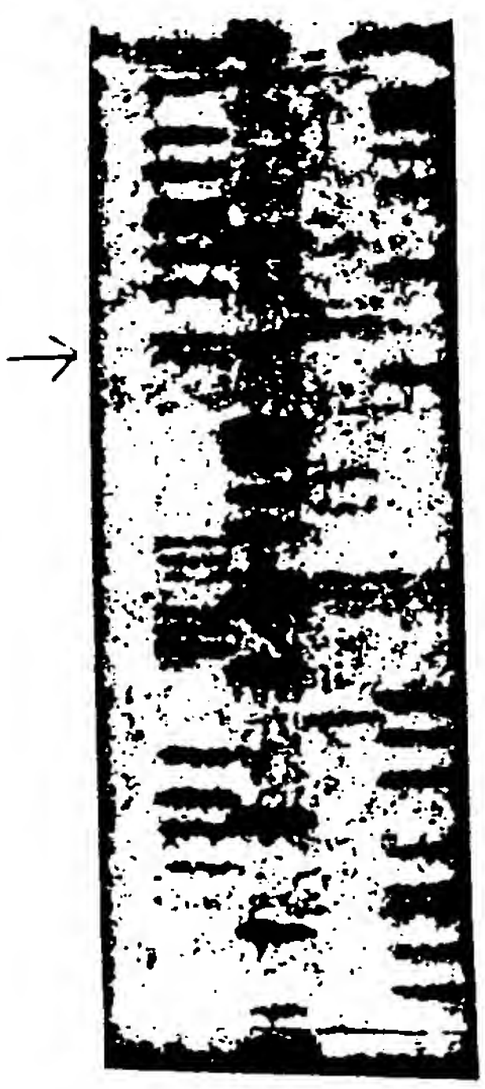
1. PCR Pert
2. PCR HPRT/αIAT/β-Globin.
3. NuSieve Gel
4. Probe with internal oligo's. (Gel or dot blot)

DNA sequencing ladders of wild type (+) and sparse fur (spf) OTC cDNA constructs, showing the spf mutation; a C to A transverson at position 348. The templates were 'full length' OTC cDNA's, cloned into the eukaryotic expression vector p91023B. Sequencing was by the method of Chen and Seeburg (1985, DNA 4, 165) using double stranded DNA as a template. The same oligonucleotide primer used in the PCR reaction to isolate the spf mutation was employed for the sequencing of this construct. The DNA strand shown is the - strand, i.e. complementary to the cDNA strand shown in fig. one of the manuscript. The order of the sequencing tracks is T, C, A, G.

+

spf





Joel Ramón

EV-_9D5'

10	20	30	40	50	60
ACCCAAATAC	TTTGTTCATG	TTTAAATTTT	ACAACATTTC	ATAGACTATT	AAACATGGAA
70	80	90	100	110	120
CATCCTTGTG	GGGACAAGAA	ATCGAATTTG	CTCTTGAAAA	GGTTTCCAAC	TAATTGATTT
130	140	150	160	170	180
GTAGGACATT	ATAACATCCT	CTAGCTGACA	AGCTTACAAA	AATAAAAACT	GGAGCTAACC
190	200	210	220	230	240
GAGAGGGTGC	TTTTTTCCCT	GACACATAAA	AGGTGTCTTT	CTGTCTTGTA	TCCTTTGGAT
250	260	270	280	290	300
ATGGGCATGT	CAGTTTCATA	GGCAAATTTT	CACATGGAGC	TTTTGTATTT	CTTTCTTTGC
310	320	330	340	350	360
CAGTACAACT	GCATGTGGTA	GCACACTGTT	TAATCTTTTC	TCAAATAAAA	AGACATGGGG
370	380	390	400	410	420
CTTCATTTTT	GTTTTGCCTT	TTTGGTATCT	TACAGGAACT	CCAGGATGGC	ATTGGGCAGC
430	440	450	460	470	480
GGCAAACTGT	TGTCAGAACA	TTGAATGCAA	CTGGGGAAGA	AATAATTCAG	CAATCCTCAA
490	500	510	520	530	540
[illegible]ACAGATGC	CAGTATTCTA	CAGGAAAAAT	TGGGAAGCCT	GAATCTGCGG	TGGCAGGAGG
550	560	570	580	590	600
TCTGCAAACA	GCTGTCAGAC	AGAAAAAAGA	GGTAGGGCGA	CAGATCTAAT	AGGAATGAAA
610	620				
ACATTTTAGC	AGACTTTTTA	AGCTT			

clone

ratio OD_{610} @ 30' $\left(\frac{pH\ 7.7}{pH\ 10.0}\right)^{-1}$ 0.80 4.3 0.57

spf control

(+) pH 7.7

(+) pH 10.0

spf pH 10.0

spf pH 7.7

(-) pH 7.7

(-) pH 10.0

OD_{610}

1.2

1.0

0.8

0.6

0.4

0.2

0

10

20

30

time (mins)

α [Citrulline]:

[redacted]/7 Titering the Human Genomic Library (David Nelson)

- titer thought to be 2×10^{10}
- want 50-100 plaques
- 50 → 1×10^3/ml
  but titer thought to be 2×10^{10}
  50 → 2×10^3/ml

10^{10}
↓ 1/100
10^8
↓ 1/100
10^6
↓ 1/100
10^4
↓ 1/10
10^3

⇒ $1/10^7$ dilution

(50 λ of 1/10⁷) (½ of what expected, 2×10^{10})

$\underline{46} \times 20 = 9.20 \times 10^2$/ml $\times 10^7 = 9.2 \times 10^9$/ml

use $1/10^4$ dilution [titer 9.2×10^5]

$5 * 10^4 \div 9.2 \times 10^5 = .054 = 54$ 3µl

$4 \times 10^4 \div$ " $= .0435 = 43.5$µl

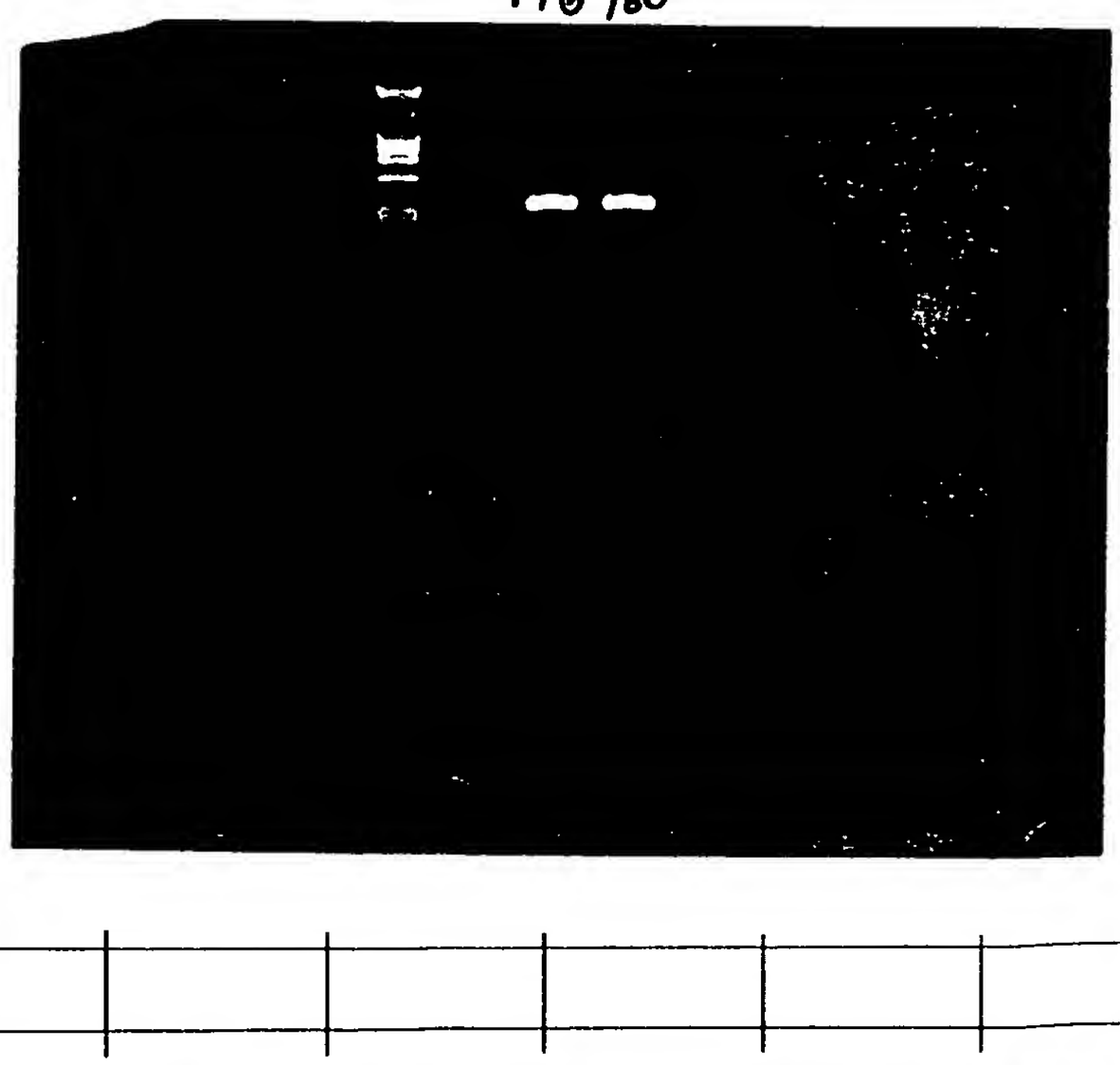
X take 1/10² take 20 µl into 2ml
used all of $1/10^4$ dilution ≅ 990µl

Joel Ranier

PCR to Test Oligo's 251, 252 (#1) 3/4/18

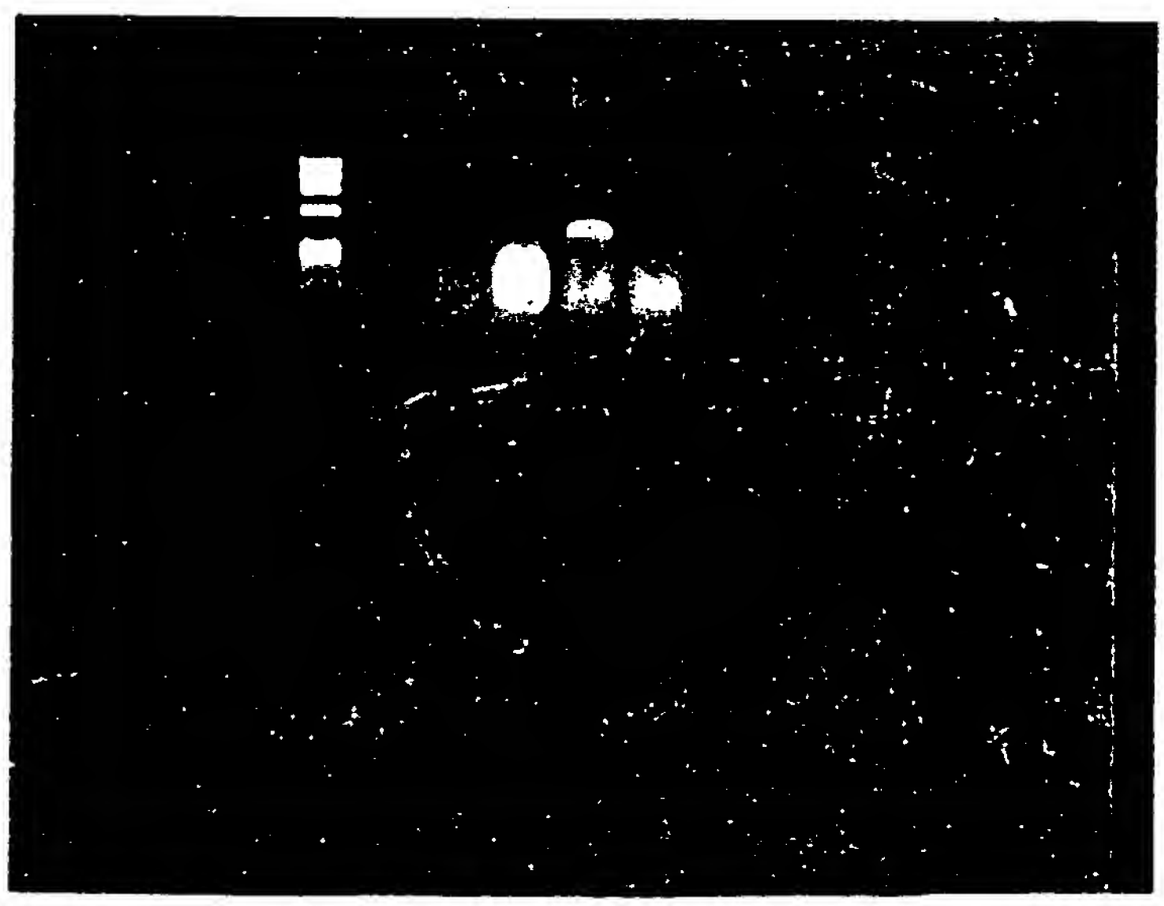
	1	2	3	(+) control	(+) control	6	(-) control	8	9	
1	template			776	no template		780			
2				0.5 µl			0.8 µl			
3	primer 25			0.5	0.5		0.5			
4	(198 µM)			(1 µM)						
5	primer 25			0.8	0.8		0.8			
6	(129 µM)			(µM)						
7										
8	5x Taq buff			20	20		20			
9										
10	dNTP			6	6		6			
11										
12	dH_2O			62.2	62.7		61.9			
13										
14	DMSO			10	10		10			
15				100						
16										
17	94°C	7' (fg 3 sec, .5 Taq								
18	37°C	30 sec								
19	65°	2'								
20										
21	60 mJ	1% TBE								
22										
23	94°C	1'								
24	37°C	30"								
25	65°	1'								
26										
27	//// //// ////									
28	//// //// ////									
29	//// ////									
30										
31										

776 780 –



PCR (second) C. primers 251, 252) to amp Mande exon 3/7/88

	1	2	3	(+) control	(+) control	(-) control	(-) control	8	9
1	Template			776	776	no template	158	(deleted)	
2				0.5 λ	0.5λ	—	λ	0.6 µg/ml	
3	primer 251			0.5λ	0.5λ	0.5 λ	0.5λ		
4	(198 µM)			(1 µM)					
5	primer 252			0.8 λ	0.8λ	0.8λ	0.8λ		
6	(129 µM)			(1 µM)					
7	primer 221			1.1λ	—	—	— λ		
8	(94 µM)			(1.0 µM)					
9	primer 222			.83 λ	—	—	—		
10	(121.7 µM)			(1.0µM)					
11	5x Taq			20	20	20	20		
12									
13	dNTP			6	6	6	6		
14	2.5mM								
15	dH_2O			52.8	62.2	62.7	61.7		
16									
17	DMSO			10	10	10	10		
18				100λ					
19	94° 1' cfg 3sec								
20	.5λ Taq			loaded 776 (1 set), 776 (2 sets), 158 del., no temp.					
21	37° 30sec								
22	65° 2'								
23									
24	94 1'								
25	37° 30sec								
26	65° 1'								
27									
28									
29									
30									
31									



PCR #3

3/9/8

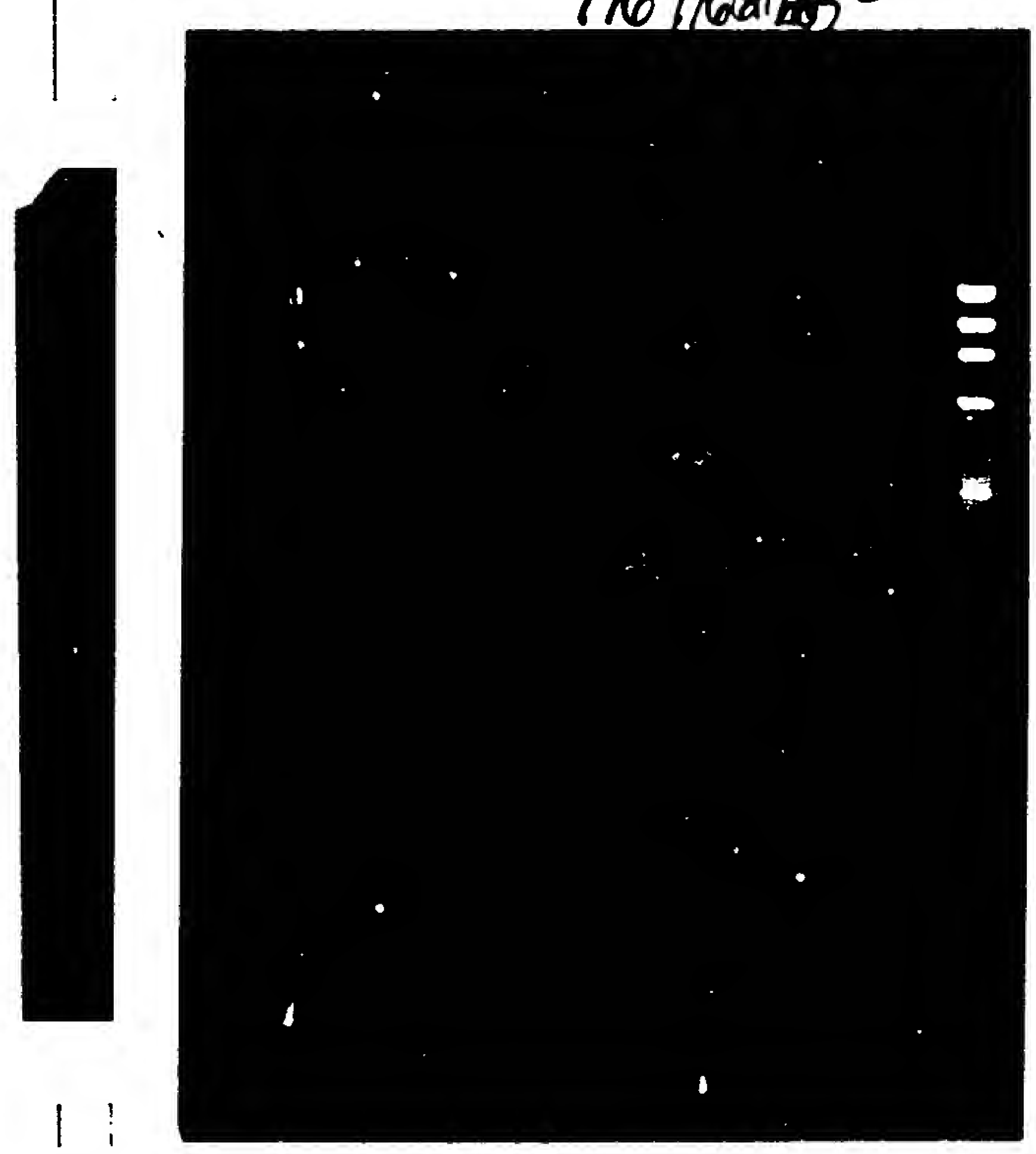
1	2	(+)control	(+)control	(−)control	(−)control	(−)control	8	9
template		776 0.5λ	776 0.5λ	641 0.25λ	665 2.5λ	— —		
primer	251	0.5λ (1µM)	0.5λ	0.5λ	0.5λ	0.5λ		
primer	252	0.8λ	0.8λ	0.8λ	0.8λ	0.8λ		
primer	221	1.1λ	—	—	—	—		
primer	222	0.85λ	—	—	—	—		
5xTaq		20	20	20	20	20		
dNTP		6	6	6	6	6		
dH_2O		60.2	62.2	62.4	60.2	62.7		
DMSO		10	10	10	10	10		

776 1µg/ml
641 2.15µg/ml
665 .2µg/ml

94°C 7' dg 3sec
37°C 30s
65°C 2'

(diff. enz. nec's)
~~HH~~ ~~HH~~ 94°C 1'
~~HH~~ ~~HH~~ 37°C 30s
~~HH~~ ~~HH~~ 65°C 1'
~~HH~~ IIII

776 776 641 665 −



PCR #4 (To test double primer set) 3/10/8

Mix for 3 rxn's (w/o) oligo's

	For 1 RXN	For 3 RXN's
template (776)	4λ	12λ
5x Taq	20λ	60λ
dNTP	6λ	18λ
dH_2O	56.80λ	170.3λ
DMSO	10λ	30λ

Add 96.77 to 3 tubes

	776 (2 sets)	776 (1st set)	776 (2nd set)
H_2O	—	2.0λ	1.3λ
pr. 25 1	0.5λ	0.5λ	—
pr. 25 2	0.8λ	0.8λ	—
pr. 22 3	1.1λ	—	1.1λ
pr. 22 3	0.83λ	—	0.83λ

No Amplification

Joel Rainer

PCR #5 (c new enzyme)

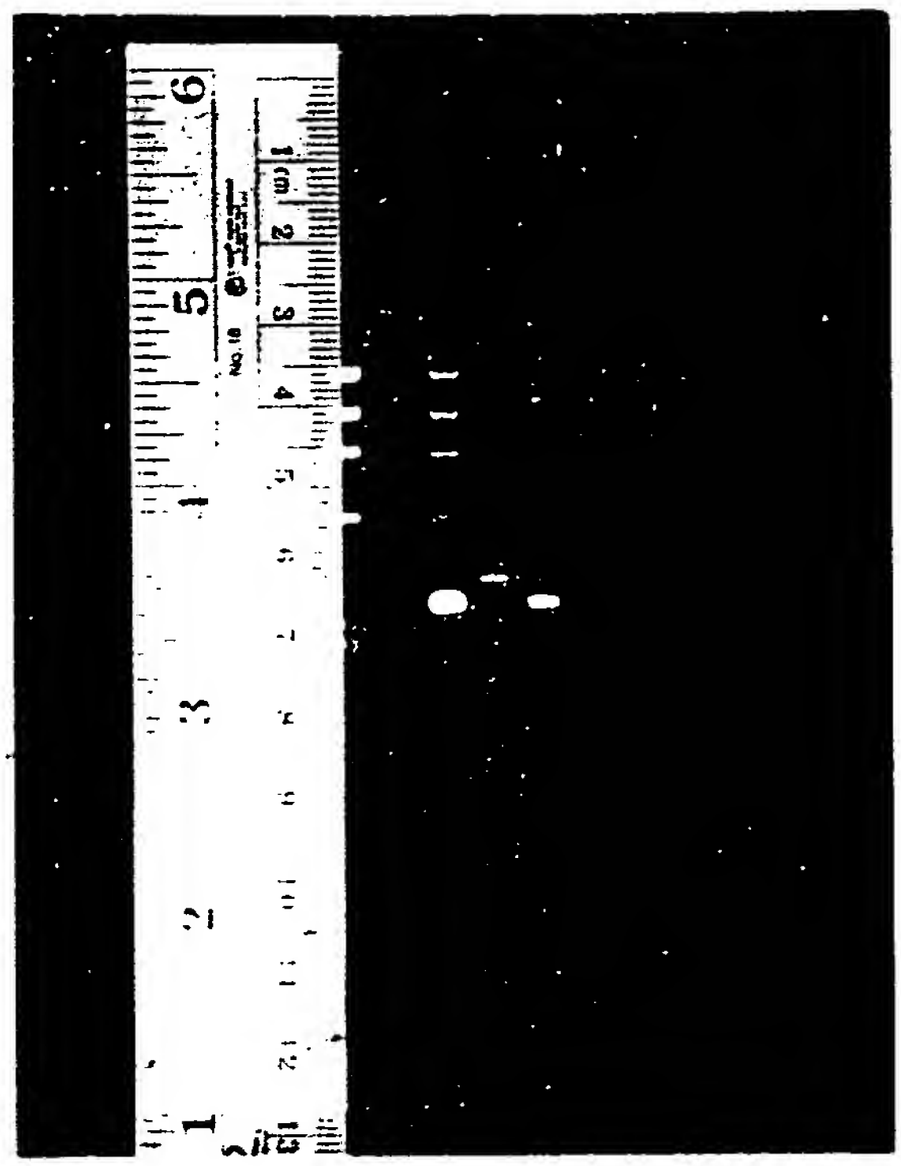
3/17/8

Mix for 3 rxns (w/o oligo's)

	For 1 RXN	For 3	For no template
template (776 dil)	4		
5x Taq	20		
dNTPs	6		
dH_2O	56.8		
DMSO	10		
Add 96.8 to each tube			
	776 (1st set)	776	
(2 sets)			
Nr. 251	0.5	0.5	
252	0.8	0.8	
221	1.1	—	
222	0.8	—	
H_2O	—	2.0	

94°C 1' cfg 3sec
37°C 30sec
Add enz.
65° 2'
94°C 1'
37°C 30sec
65°C 1'30"

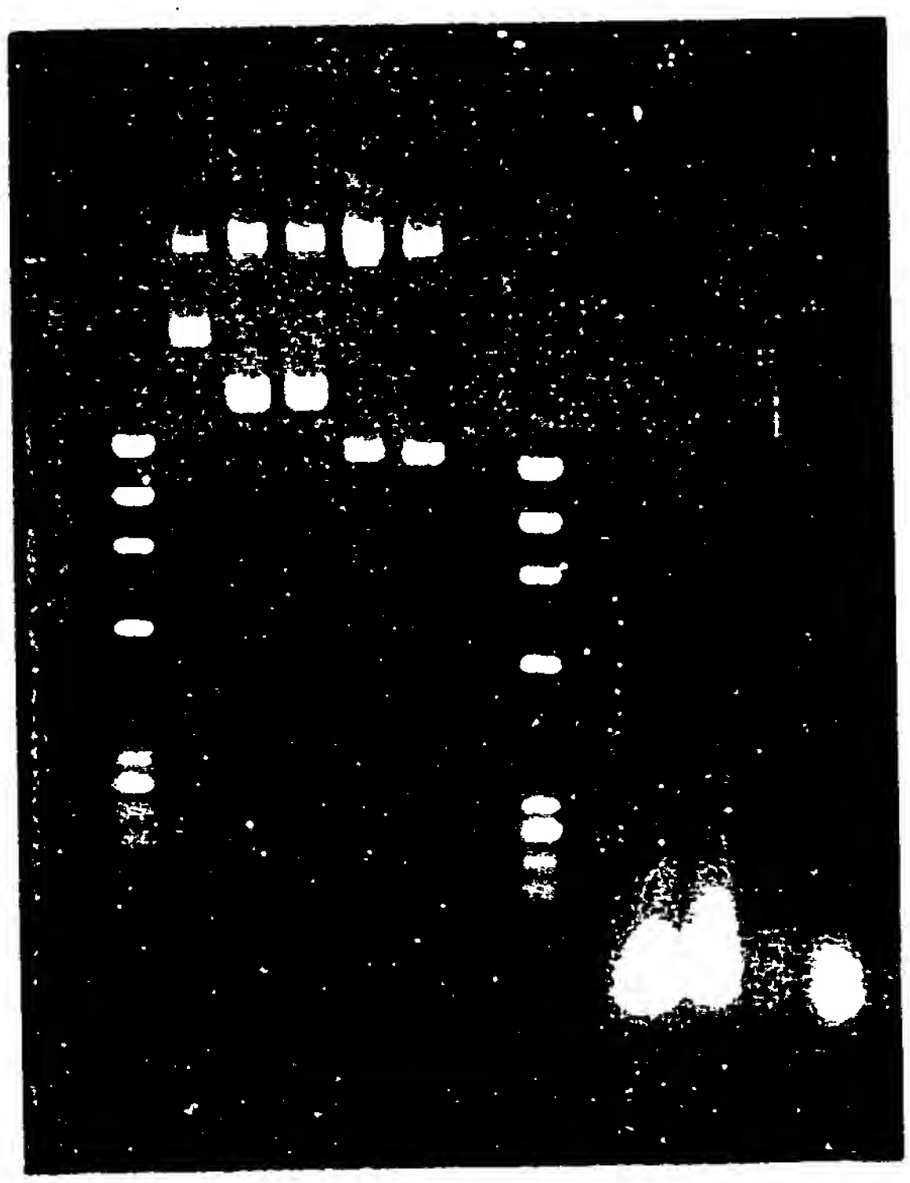
776 1st set 2nd set

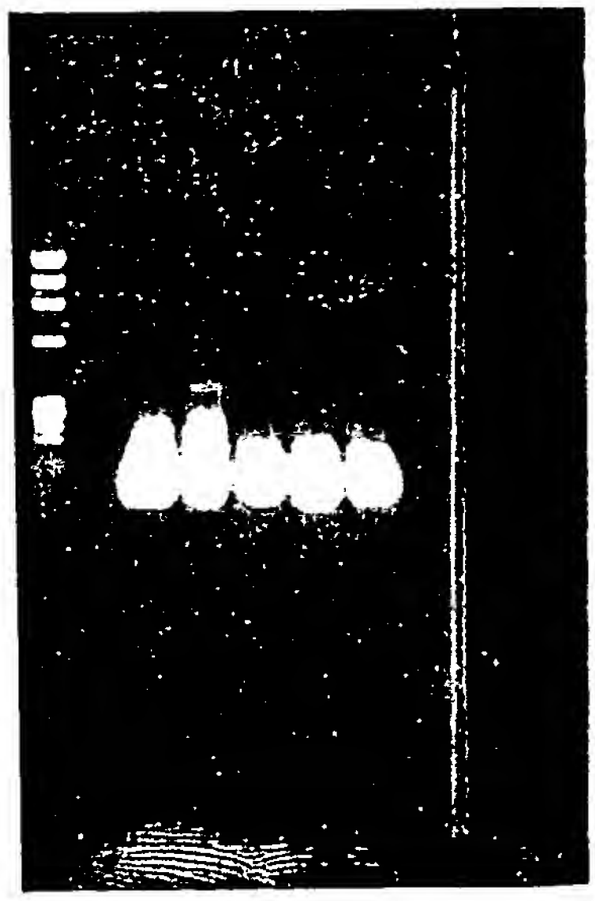


PCR #6 (to test variables) 3/21/8

	1	2	3	4 776	5 776	6 776	7 776	8 776	9
1	template (776)			4	4	4	4	4	
2									
3									
4	Primer	221		1.6	1.1	1.1	1.1	1.1	
5		222		~~0.8~~ 1.1	0.8	0.8	0.8	0.8	
6		251		0.25	0.5	0.5	0.5	0.5	
7		252		0.40	0.8	0.8	0.8	0.8	
8									
9	dNTP's			6	6	12	6	6	
10									
11	5x Taq			20	20	20	20	20	
12									
13	DMSO			10	10	10	10	10	
14									
15	H_2O			56.65	56.8	50.8	56.8	56.8	
16						100			
17	Temp. Variable								
18									
19	(4) Annea								
20	(5) "								
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

EFFICIENCY LINE = 22-206

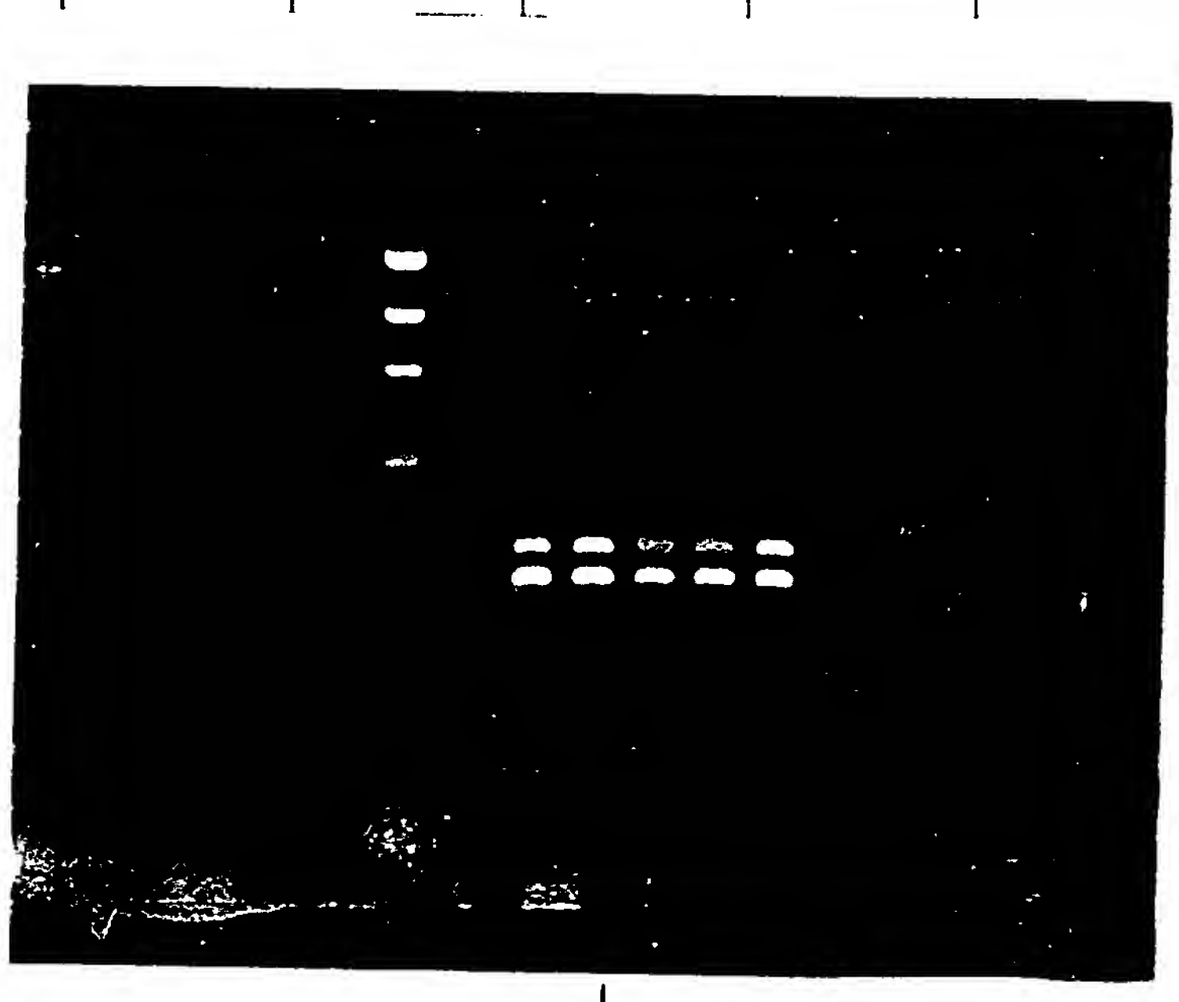




Joel Ranier

PCR #7 (To test new nuc's, enz, stringency) 3/23/8

	1	2	3	4	5	6 √2-7	7	8	9	
1					776	776	776	776	776	
2	template				4	4	4	4	4	
3										
4	primer	221	(48 μM)		2	2	2	2	2	
5		222	(56 μM)		1.8	1.8	1.8	1.8	1.8	
6		251	(198 μM)		0.5	0.5	0.5	0.5	0.5	
7		252	(129 μM)		0.8	0.8	0.8	0.8	0.8	
8										
9	dNTP's				6 old	6 new	6 old	6 new	6 new	
10					(old enz)	(new enz)	(new enz)	(old enz)	(new enz)	
11										
12	5x Taq				20	20	20	20	20	
13										
14	DMSO				10	10	10	10	10	
15										
16	H_2O				54.9	54.9	54.9	54.9	54.9	
17										
18									450	
19										
20										
21										
22										
23										
24										
25										
26										
27										
28	//// ////									
29	//// ////									
30	//// ////									
31	//// ////									



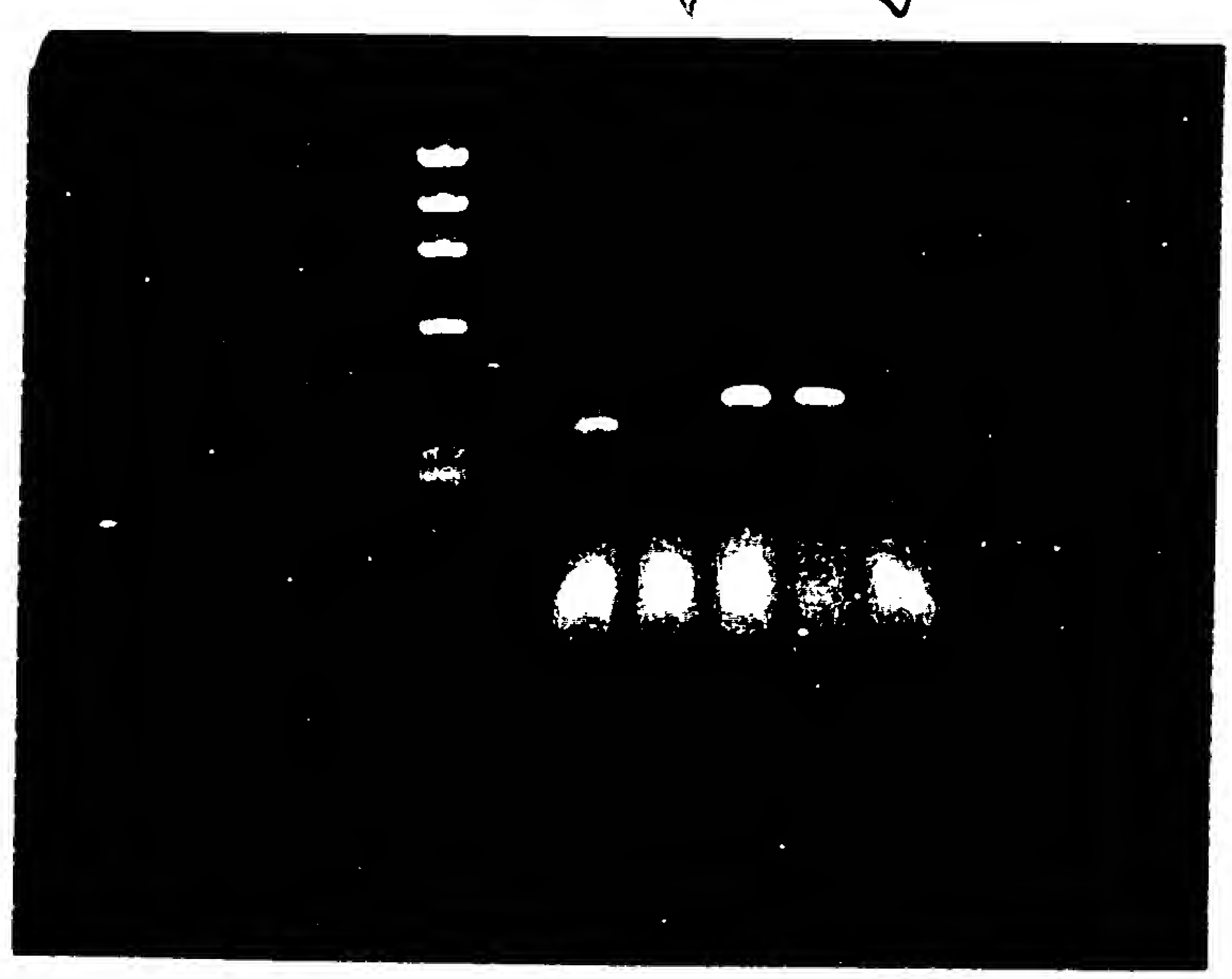
TOPS FORM 3619 LITHO U.S.A.

PCR #8 (To test diagnostic cases) 3/24/8

1	2	3	4	5 776	6 JB	7 665	8 680	9 —
				normal	double deletion	42 -75, +87	+75-81 H addicion	no
template				4 (.125 μg/λ)	1.67 (0.3 μg/λ)	2.5 (0.2 μg/λ)	0.66 (0.76 μg/λ)	template
primer	221	(48 μM)		2	2	2	2	2
	222	(56 μM)		1.8	1.8	1.8	1.8	1.8
	251	(198 μM)		0.5	0.5	0.5	0.5	0.5
	252	(129 μM)		0.8	0.8	0.8	0.8	0.8
dNTP's				6	6	6	6	6
5xTaq				20	20	20	20	20
DMSO				10	10	10	10	10
H_2O				54.9	57.2	56.4	58.24	58.9
						100 μl		

94°C 7' cfg
37°C 30"
.5λ Taq Pol

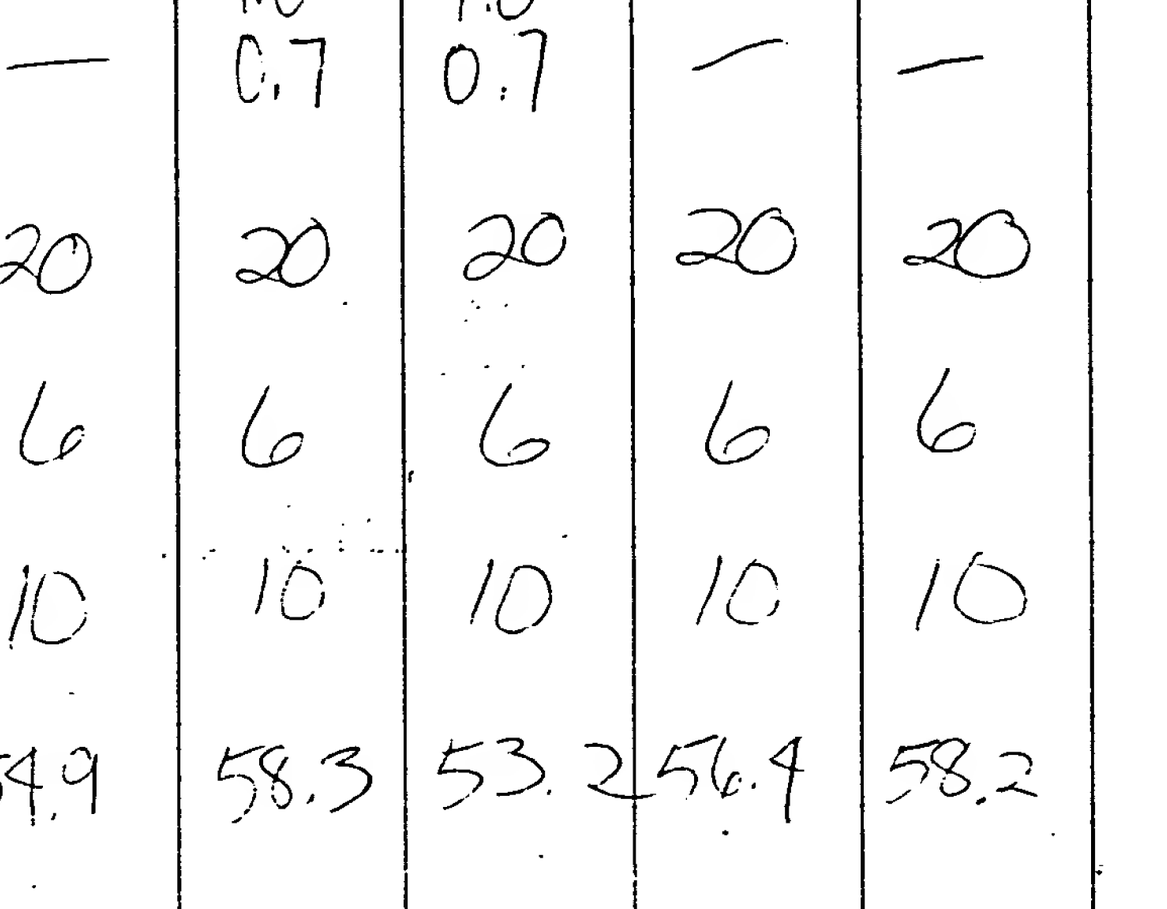
√ √



Annealed at 40°C

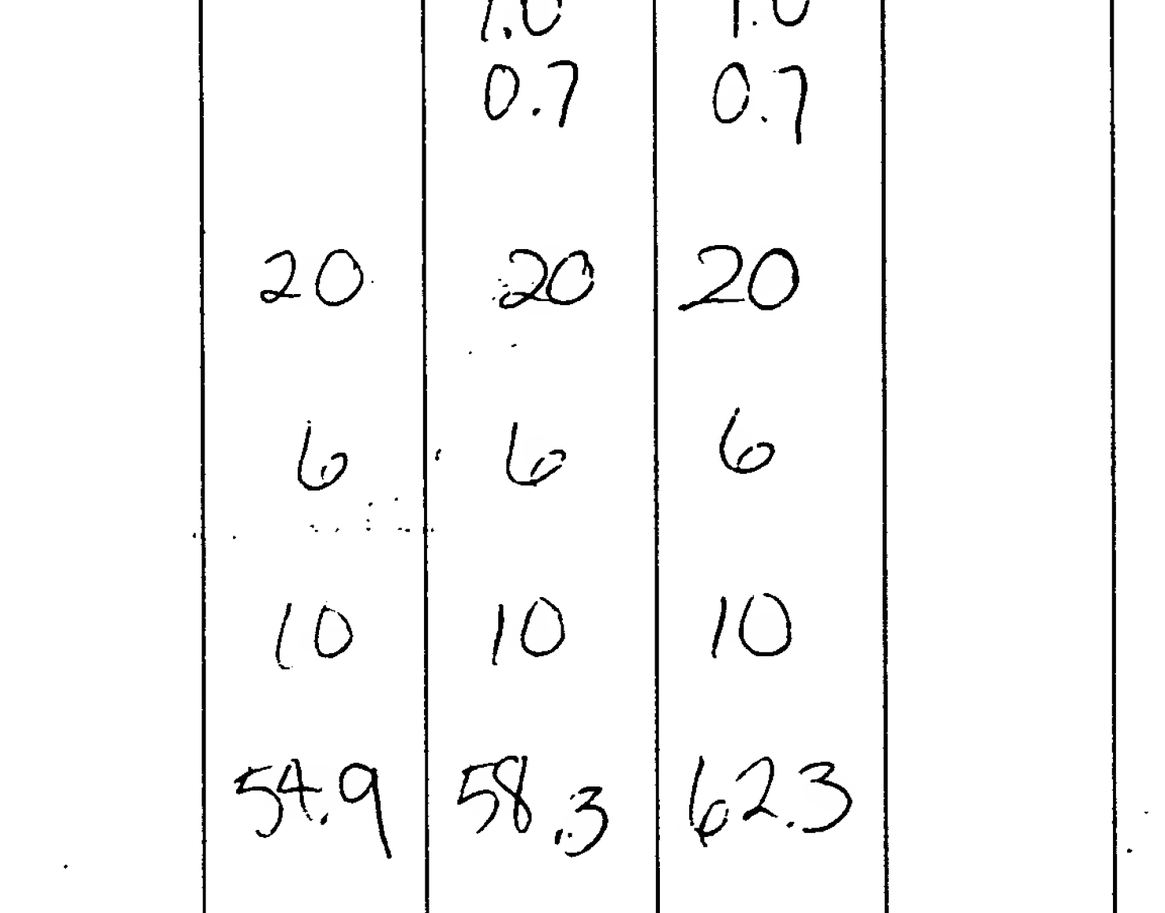
PCR #9 (to test 3rd set oligo's + diagnostic 3/27/8

	1	2	3	4	5 (use set)	6	7	8	9
1				4λ	4λ	4λ	2.5λ	0.7λ	
2	template			776 (2 sets)	776 (new set)	776 (all sets)	665 (2 sets) -75, +87	660 (2 sets) +87, -87	
3					—				
4	primer	221	(48μM)	2		2	2	2	
5		222	(56μM)	1.8		1.8	1.8	1.8	
6		251	(198μM)	0.5		0.5	0.5	0.5	
7		252	(129μM)	0.8		0.8	0.8	0.8	
8		276	(106μM)	—	1.0	1.0	—	—	
9		277	(143μM)	—	0.7	0.7	—	—	
10									
11	5X Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			54.9	58.3	53.2	56.4	58.2	
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28	//// ////								
29	//// ////								
30	//// ////								
31	//// ////								



PCR #10* || (To test new oligo's from 94.1) 3/28/7

	1	2	3	4	5776	6776	7	8	9
1									
2	template				4	4	—		
3									
4	primers	221			2	—	—		
5		222			1.8	—	—		
6		251			0.5	—	—		
7		252			0.8	—	—		
8		776				1.0	1.0		
9		277				0.7	0.7		
10									
11	5x Taq				20	20	20		
12									
13	dNTP's				6	6	6		
14									
15	DMSO				10	10	10		
16									
17	H2O				54.9	58.3	62.3		
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



Joel Ranier

PCR #12 (To test assay conditions) 3/30/8

	1	2	3	4	5	6	7	8	9
1					776	776			
2	template				6	6	—		
3									
4	primers	221			2	2	~~2~~		
5		222			1.8	1.8	~~1.8~~		
6		251	(24μM)		2	~~4~~ 2	4		
7		252	(30μM)		1.7	3.4	3.9		
8									
9	5x Taq				20	20	20		
10									
11	dNTP's				6	6+6 (20)	6		
12									
13	DMSO				10	10	10		
14									
15	H_2O				50.5	46.8	~~50.8~~		
16									
17	2' extension								
18									
19	Added 4.8λ mix's at								
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

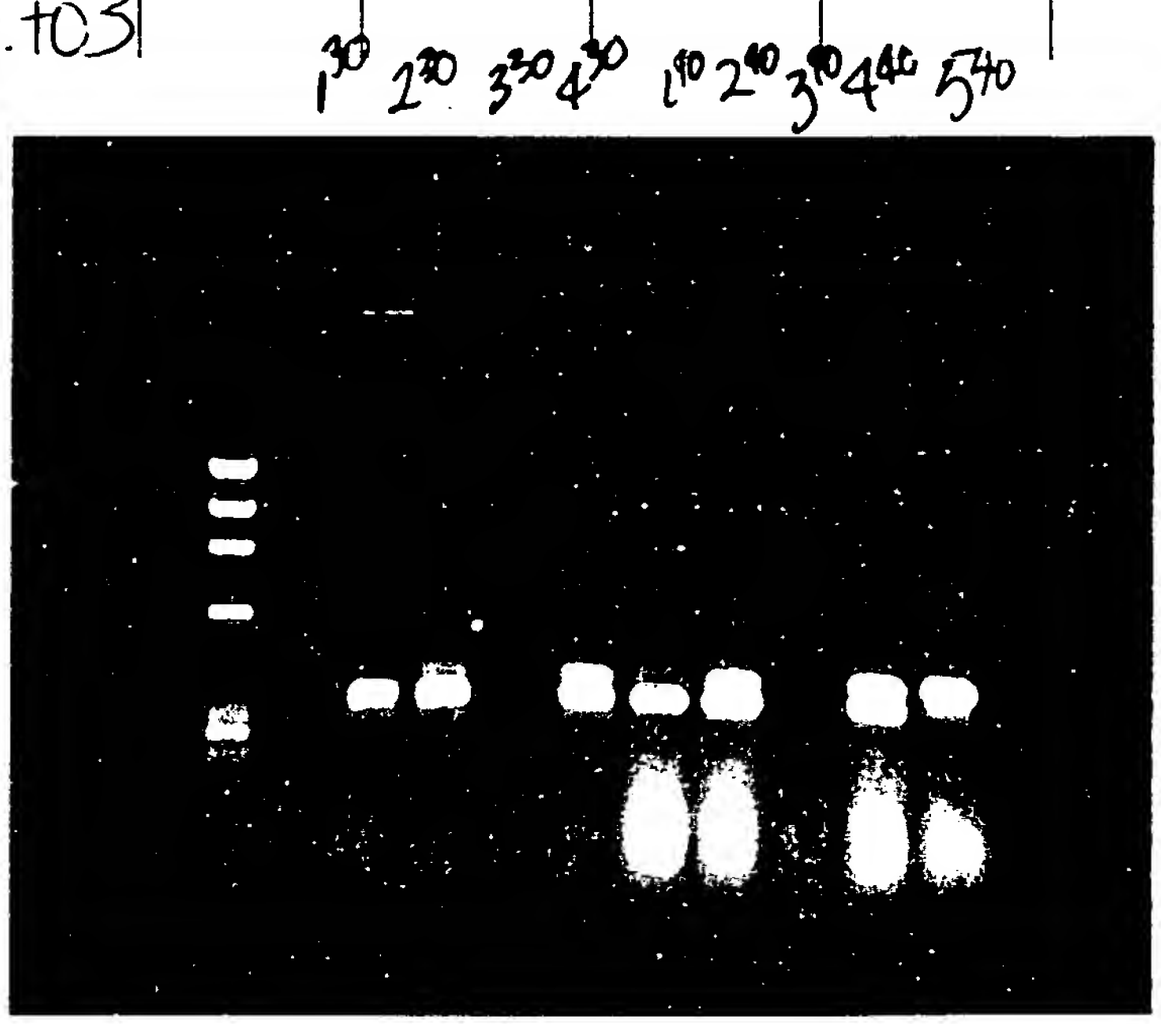
Joel Ramirez

3/31/8

PCR #13

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	776	776	776		
2	template			6	6	6	6	—	
3									
4	pr.	221		2	2	2	2	2	
5		222		1.8	1.8	1.8	1.8	1.8	
6		251		4	4	4	2	—	
7		252		3.4	3.4	3.4	1.7	—	
8									
9	5x Taq			20	20	20	20	20	
10									
11	dNTP's			6	6	6+6	6	6	
12									
13	DMSO			10	10	10	10	10	
14									
15	H_2O			46.8	46.8	46.8	50.5	60.2	
16									
17	Take out 10λ after 30rds.								
18	Add 50λ Paraffin oil to 2								
19	Add 6λ nuc's 20rds. to 3								
20	7' extension								
21									
22	Added DNA to 5?								
23									
24									
25									
26									
27									
28									
29									
30									
31									

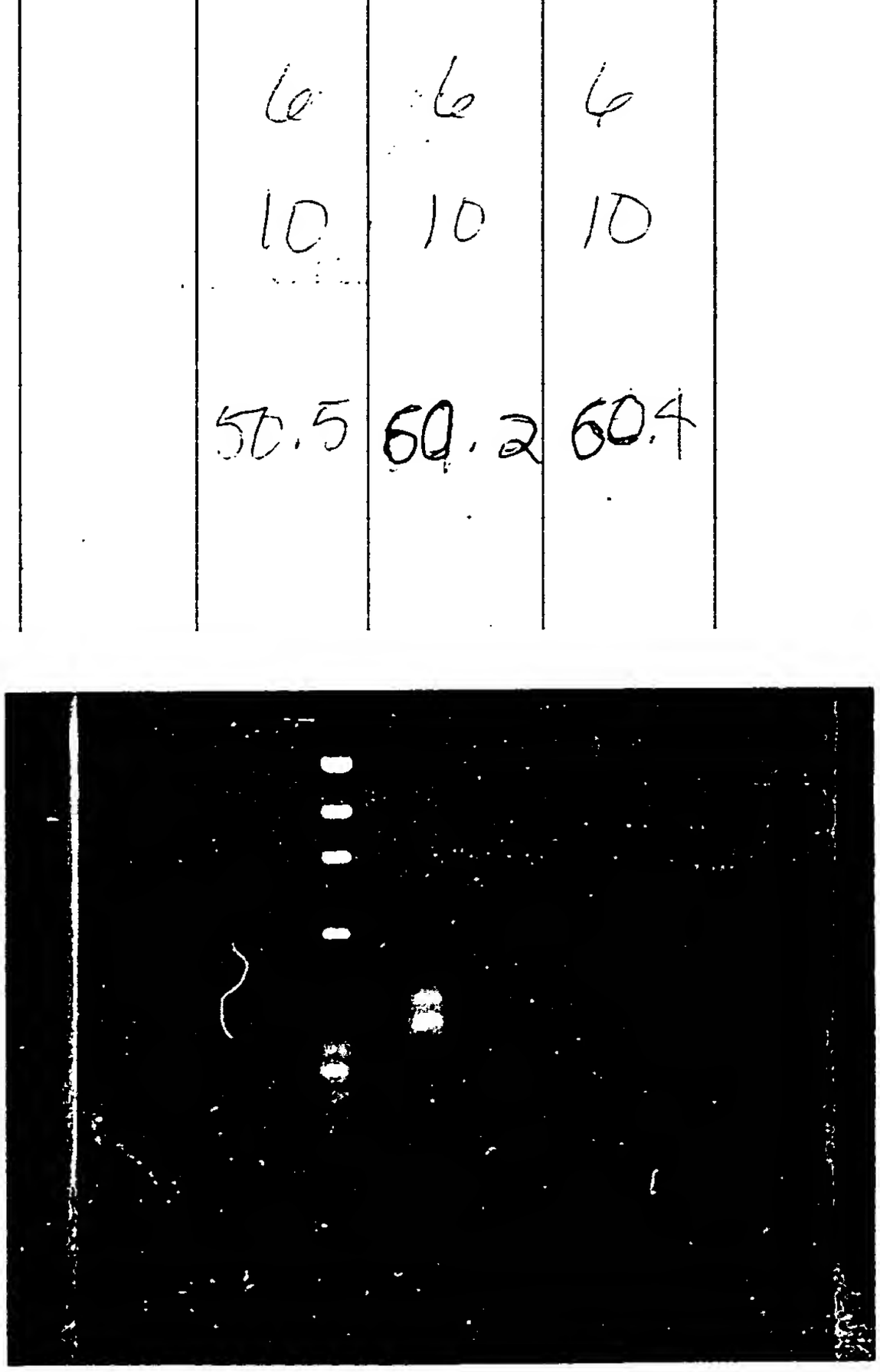
Nucs →



Joel Ranier

PCR #14 (to make sure no contamination) 4/4/87

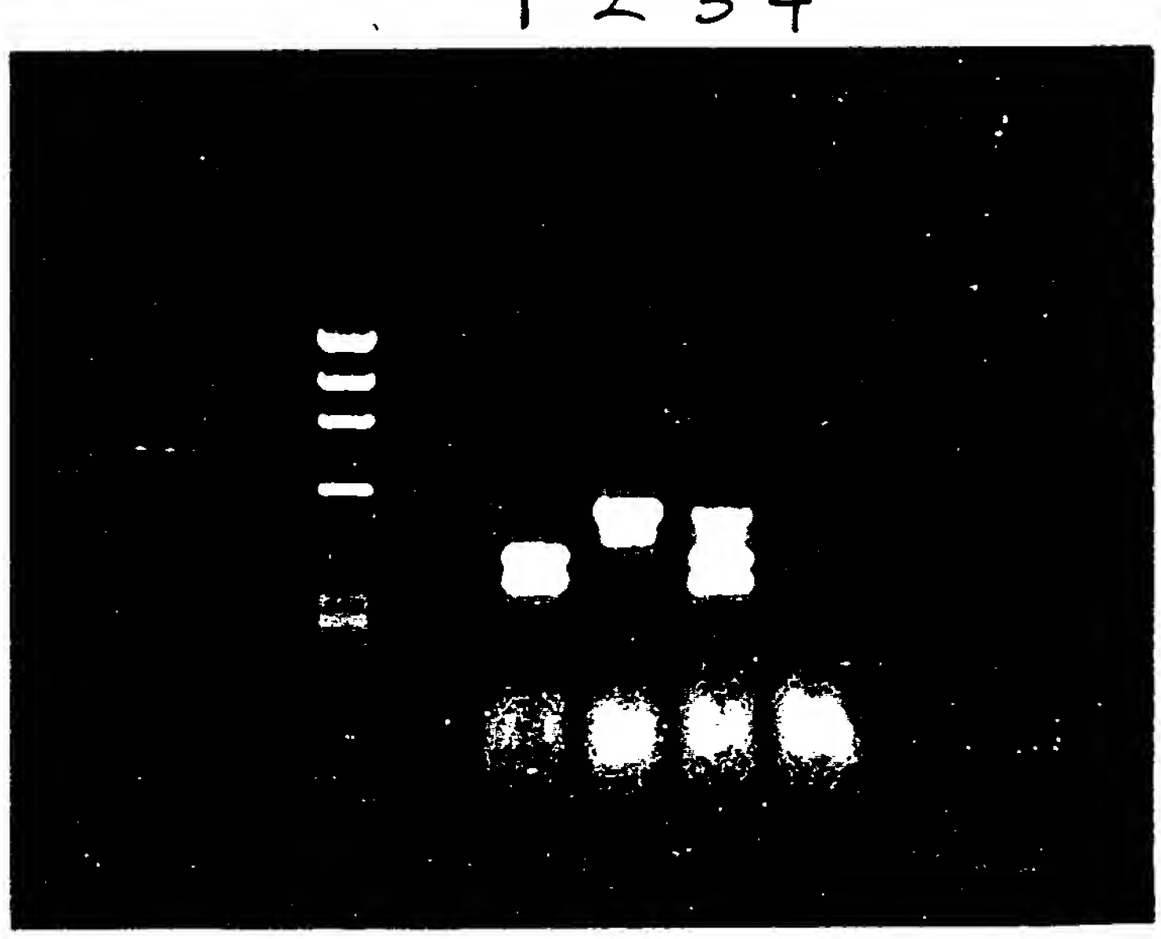
	1	2	3	4	5 776	6 776	7 776	8	9
1					6	—	—		
2	template								
3									
4	primers		221		2	2			
5			222		1.8	1.8			
6			251		2		2		
7			252		1.7		1.7		
8									
9	5xTaq				20	20	20		
10									
11	dNTP's				6	6	6		
12									
13	DMSO				10	10	10		
14	(fresh)								
15									
16	H_2O				50.5	60.2	60.4		
17									
18	2' extension								
19	30 rounds								
20	1λ Taq Pol								
21									
22									
23									
24									
25									
26									
27									
28	//// ////								
29	//// ////								
30	//// ////								
31									



Joel Ravier

PCR #15 (to test 3rd set) 4/5/8

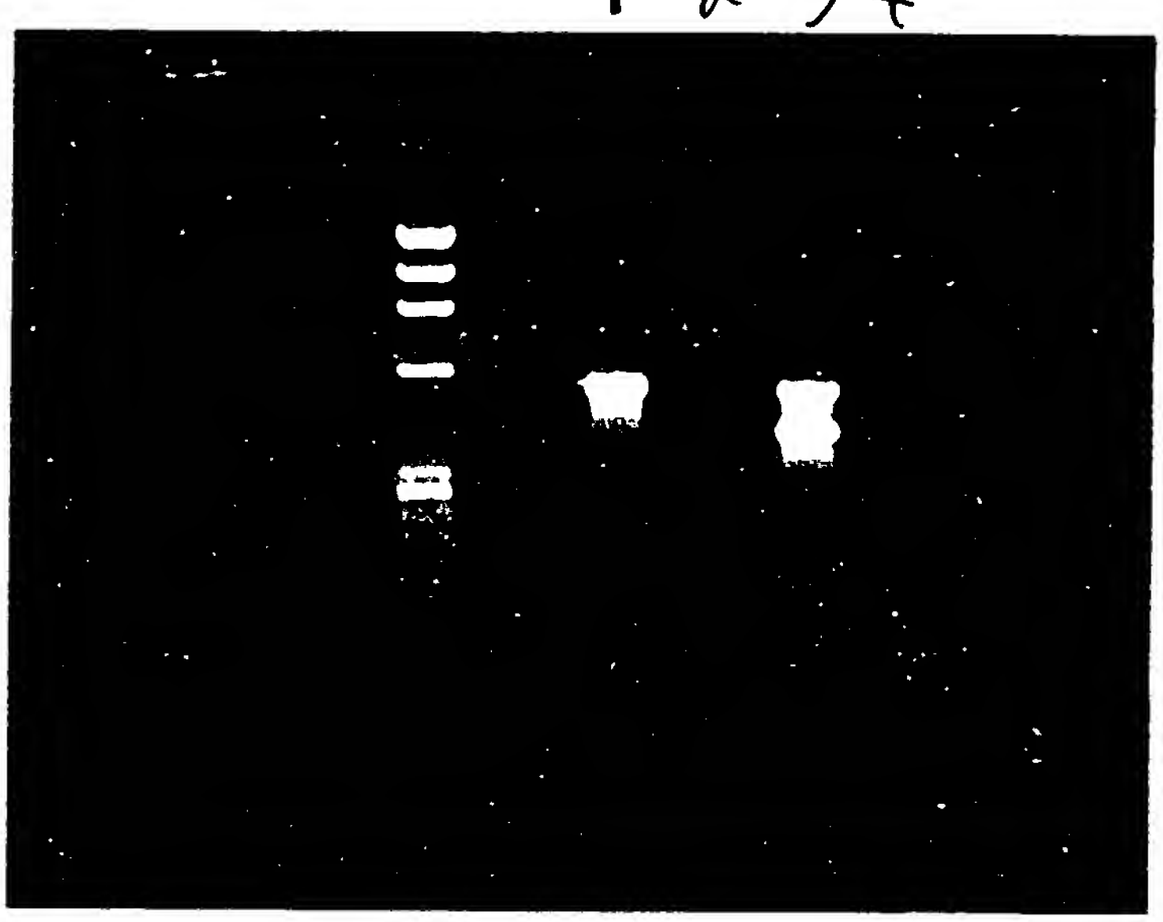
	1	2	3	4 1	5 2	6 3	7 4	8	9
1				776	776	776	—		
2	template			6	6	6			
3									
4									
5	primers	221	1 μM	2		2	2		
6		222		1.8		1.8	1.8		
7		251	0.5 μM	2		2	—		
8		252	"	1.7		1.7	—		
9		276	1 μM	—	1.0	1.0	—		
10	(44.5 μM)	303		—	2.25	2.25	—		
11									
12	5x Taq			20	20	20	20		
13									
14	dNTP's			6	6	6	6		
15									
16	DMSO			10	10	10	10		
17									
18	H_2O			50.5	~~54~~ 54.15	47.25	~~60~~ 60.2		
19						(1st 2 sets) X (new set) (all three) −			
20	2' exten.					1	2 3 4		
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



Joel Ranier

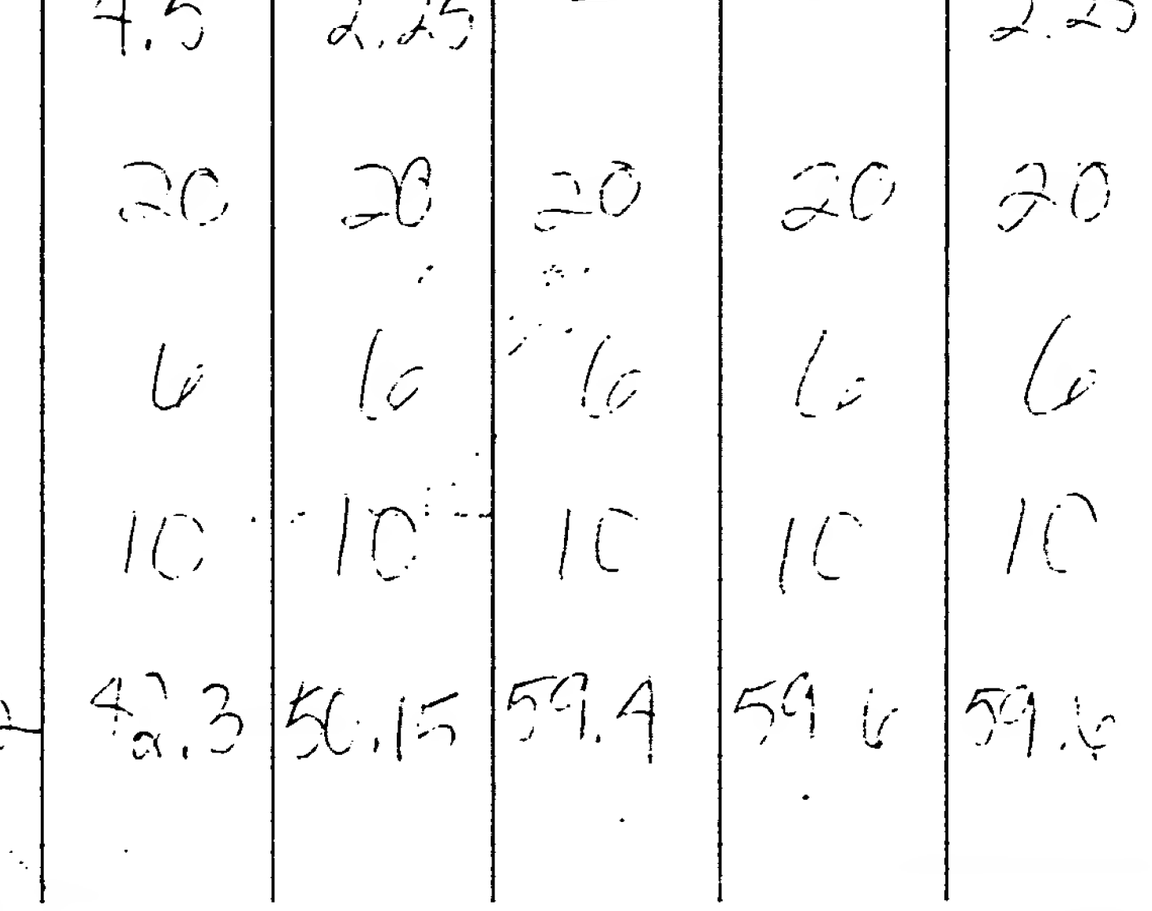
PCR #16 (to adjust stringency) 4/5/18

	1	2	3	4 1	5 2	6 3	7 4	8	9
1				776	776	776	776		
2	template			6	6	6	6		
3									
4	primers	221		—	—	2	2		
5		222		—	—	1.8	1.8		
6		251		—	—	2	2		
7		252		—	—	1.7	1.7		
8		276		1.0	1.0	1.0	1.0		
9		303		2.25	2.25	2.25	2.25		
10									
11	5x Taq			20	20	20	20		
12									
13	dNTP's			6	6	6	6		
14									
15	DMSO			10	10	10	10		
16									
17	H_2O			54.75	54.75	47.25	47.25		
18									
19	2' extension			45°C	55°C	45°C	55°C		
20						45 55 45 55			
21						1 2 3 4			
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



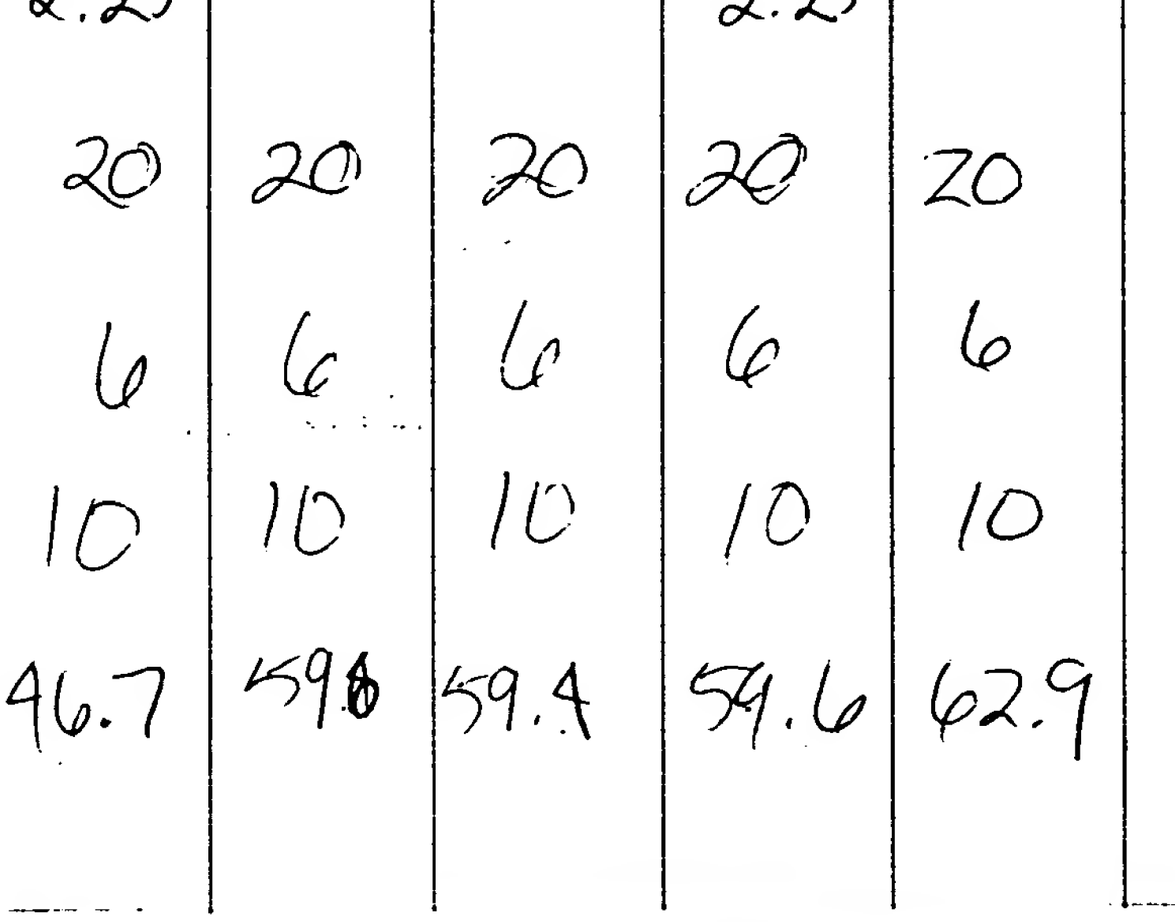
PCR #17 (to adjust oligo conc. for 3 sets) 4/7/18

	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9
1			776	776	774	—	—	—	
2	template		6	6	6				
3									
4	primers	221 (A5)	2.2	2.2	1.1	2.2	—	1	
5	(42)	222	2.4	2.4	1.2	2.4	—	1	
6	(41.8)	251	1.2	1.2	0.6	—	2.4	1	
7	(47.2)	252	1.01	1.01	0.5	—	2.0	1	
8	(45.4)	276	—	4.4	2.2	—	—	2.2	
9	(44.5)	303	—	4.5	2.25	—	—	2.25	
10									
11	5x Taq		20	20	20	20	20	20	
12									
13	dNTPs		6	6	6	6	6	6	
14									
15	DMSO		10	10	10	10	10	10	
16									
17	H2O		51.2	43.3	50.15	59.4	59.6	59.6	
18									
19	2' extension								
20	~~Pa~~ light min. oil								
21	add enz. once								
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



PCR #18 (To correct contam.) 4/9/8

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	—	—	—	—	
2	template			6					
3									
4	primers	251		1,2	2.4	—	—	—	
5		252		1.1	2.0	—	—	—	
6		221	—	2.2	—	2.2	—	0.5	(not dil.)
7		222		2.4	—	2.4	—	0.6	
8		276		2.2	—	—	2.2	—	
9		303		2.25	—	—	2.25	—	
10									
11	5x Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			46.7	45.8	59.4	54.6	62.9	
18									
19	10 rounds								
20	2' extension								
21	Anneal for 2 min								
22	1st 5 rounds								
23									
24									
25									
26									
27									
28									
29									
30									
31									

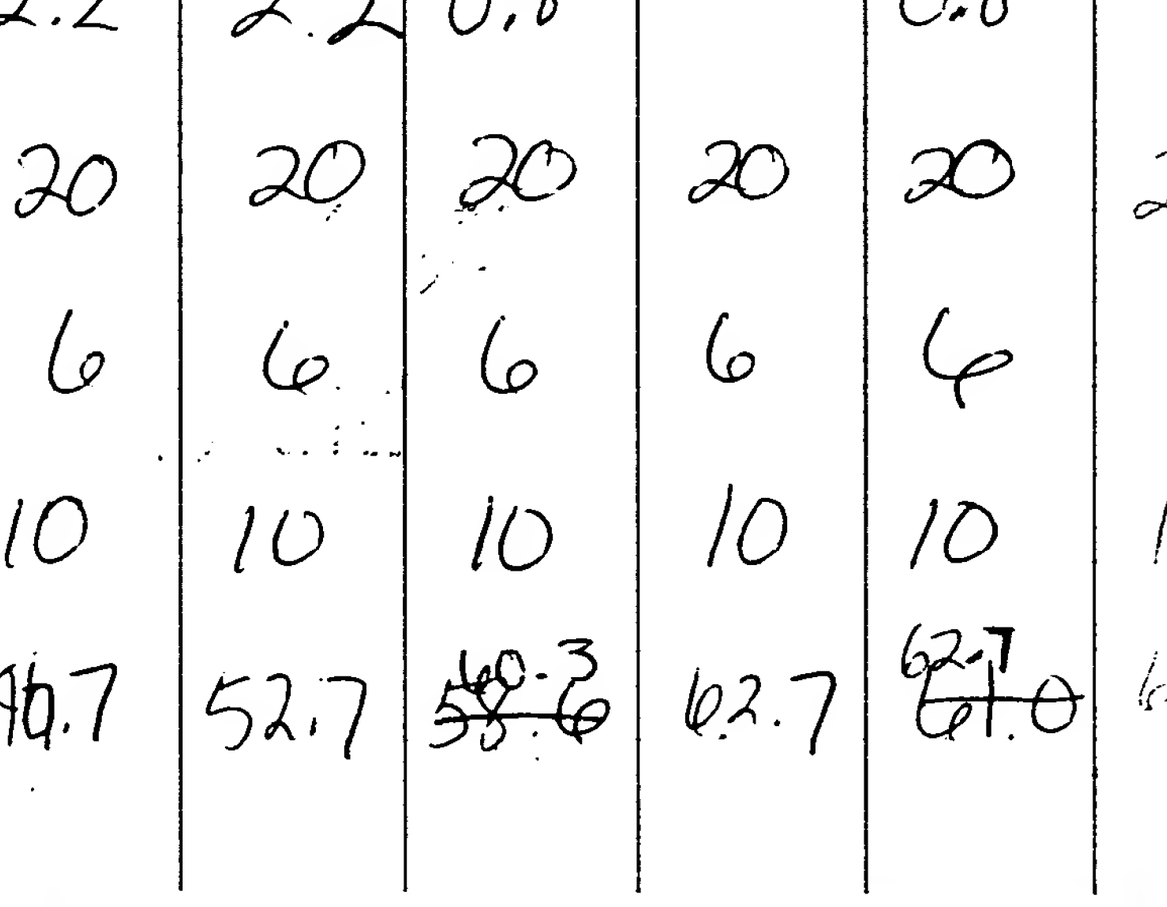


Joel Ranier

PCR #19

7/10/85

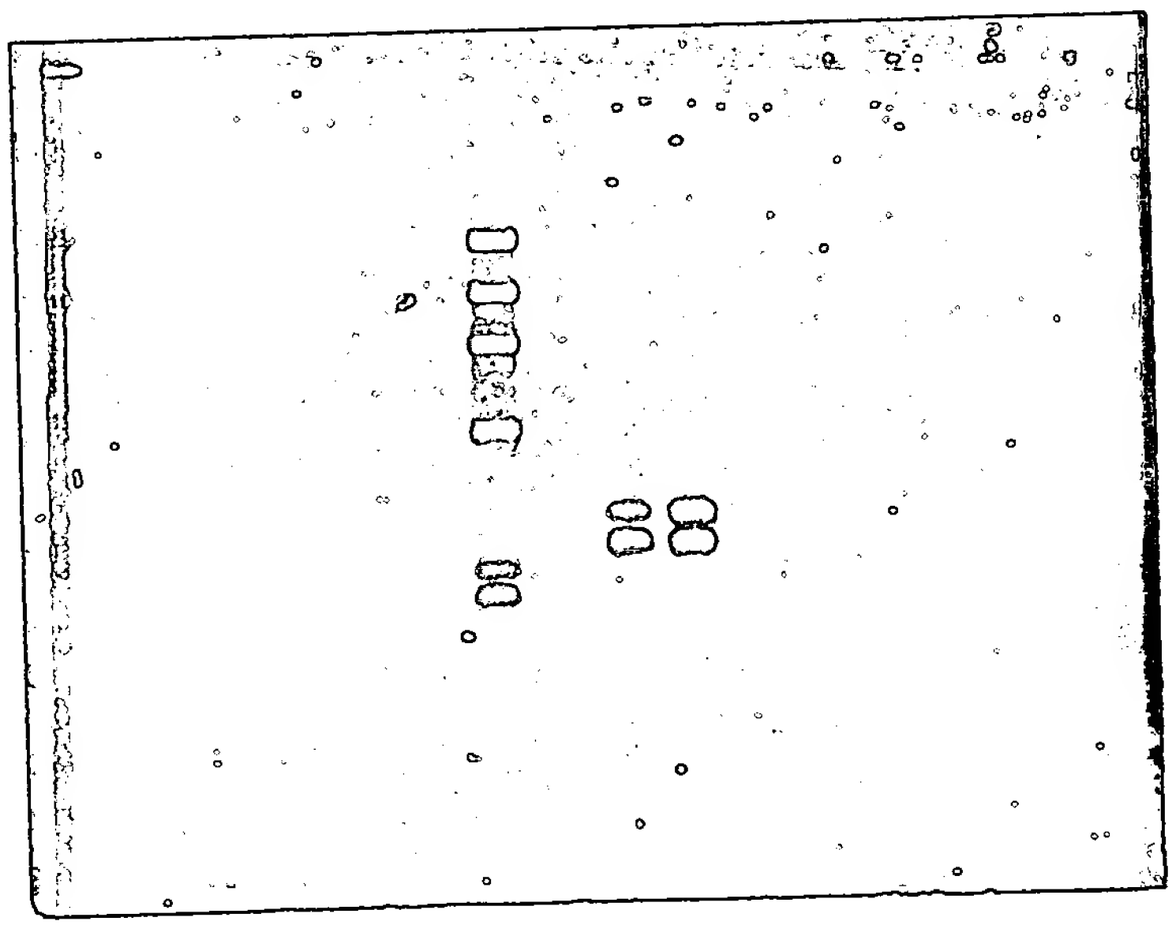
	1	2	3	4 1	5 2	6 3	7 4	8 5	9 6
1				776	—	—	—	—	—
2	template			6					
3						(no dil.)			
4	primers	221		2.2	2.2	0.5	—	—	0.5
5		222		2.4	2.4	0.6	—	—	0.6
6		251		1.2	1.2	0.5	0.5	—	—
7		252		1.1	1.1	0.8	0.8	—	—
8		276		2.2	2.2	~~2.2~~ 0.5	—	~~2.2~~ 0.5	—
9		303		2.2	2.2	0.8	—	0.8	—
10									
11	5x taq			20	20	20	20	20	20
12									
13	dNTP's			6	6	6	6	6	6
14									
15	DMSO			10	10	10	10	10	10
16									
17	H_2O			40.7	52.7	~~40.3~~ ~~58.6~~	62.7	~~62.7~~ 61.0	62.9
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



PCR #20

4/14/8

	1	2	3	4 1	5 2	6 3	7	8	9
1				776	776	—			
2	template			4	4				
3									
4									
5	Primers	221	(1μM)	2.2	2.2	2.2			
6	(42)	222	"	2.4	2.4	2.4			
7	(51.6)	251	"	1.95	1.95	1.95			
8	(208)	252	"	0.5	0.5	0.5			
9	~~(45.4)~~	276	"	2.2	2.2	2.2			
10	~~(44.5)~~	303	"	2.25	2.25	2.25			
11									
12	5x Taq			20	20	20			
13									
14	dNTP's			6	6	6			
15									
16	DMSO			10	10	10			
17									
18	H_2O			46.5	46.5	52.5			
19									
20	Use 2-25u Taq Pol			40°C	43°C	40°C			
21									
22									
23									
24									
25									
26									
27									
28									
29	← 5min 65°C								
30									
31	← 5min 65°C								



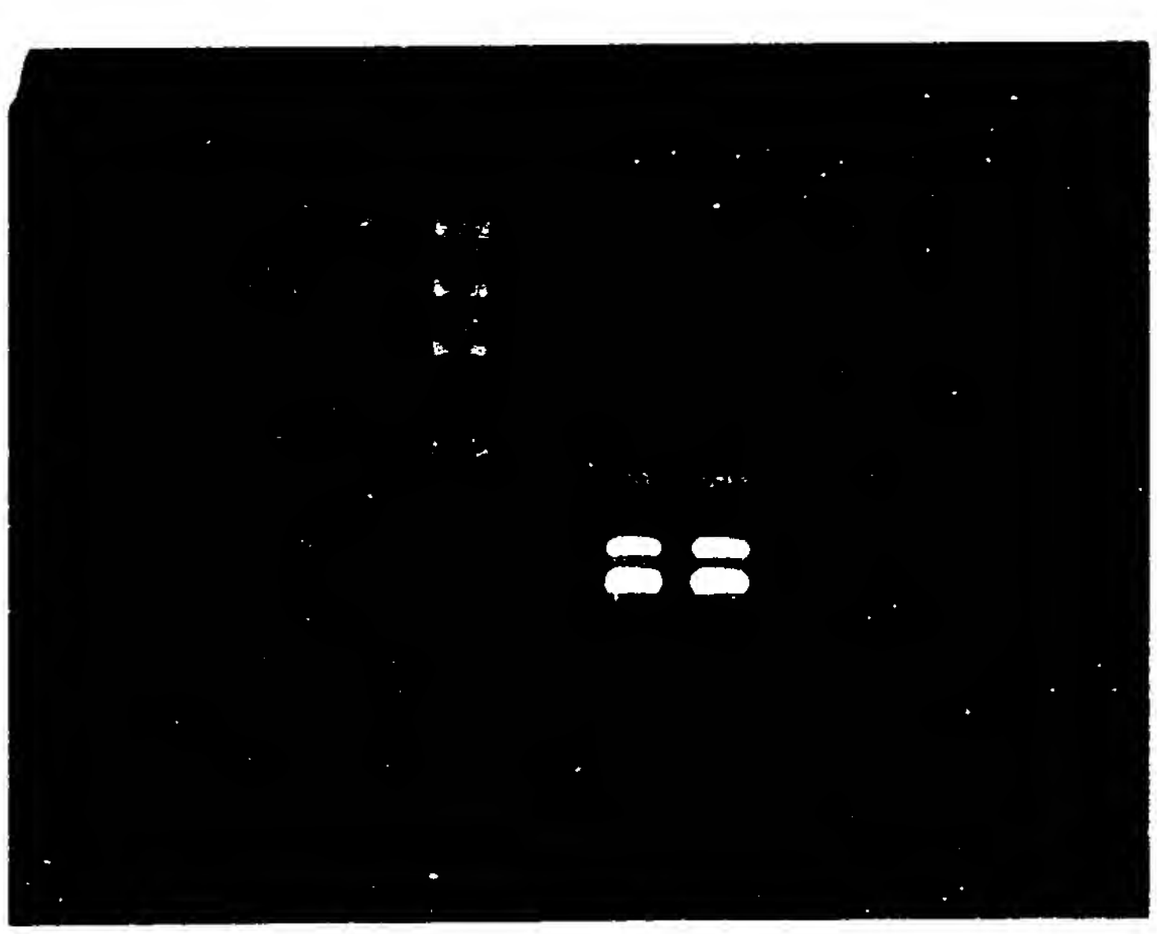
AMPAD · EFFICIENCY LINE 22-206

Joel Ranier

PCR #21

4/17/8

	1	2	3	4	5 1	6 2	7 3	8	9
1					776	776	—		
2	template				6	6			
3									
4	primers	221			2.2	2.2	2.2		
5		222			2.4	2.4	2.4		
6		251			1.95	1.95	1.95		
7		252			0.5	0.5	0.5		
8		276			2.2	2.2	2.2		
9		303			2.25	2.25	2.25		
10									
11	5x Taq				20	20	20		
12									
13	dNTP's				6	6	6		
14									
15	DMSO				10	10	10		
16									
17	H_2O				46.5	46.5	52.5		
18									
19	Used excess enz.				37°C	43°C	37°C		
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30	5 min 95°								
31		extend 5 min							

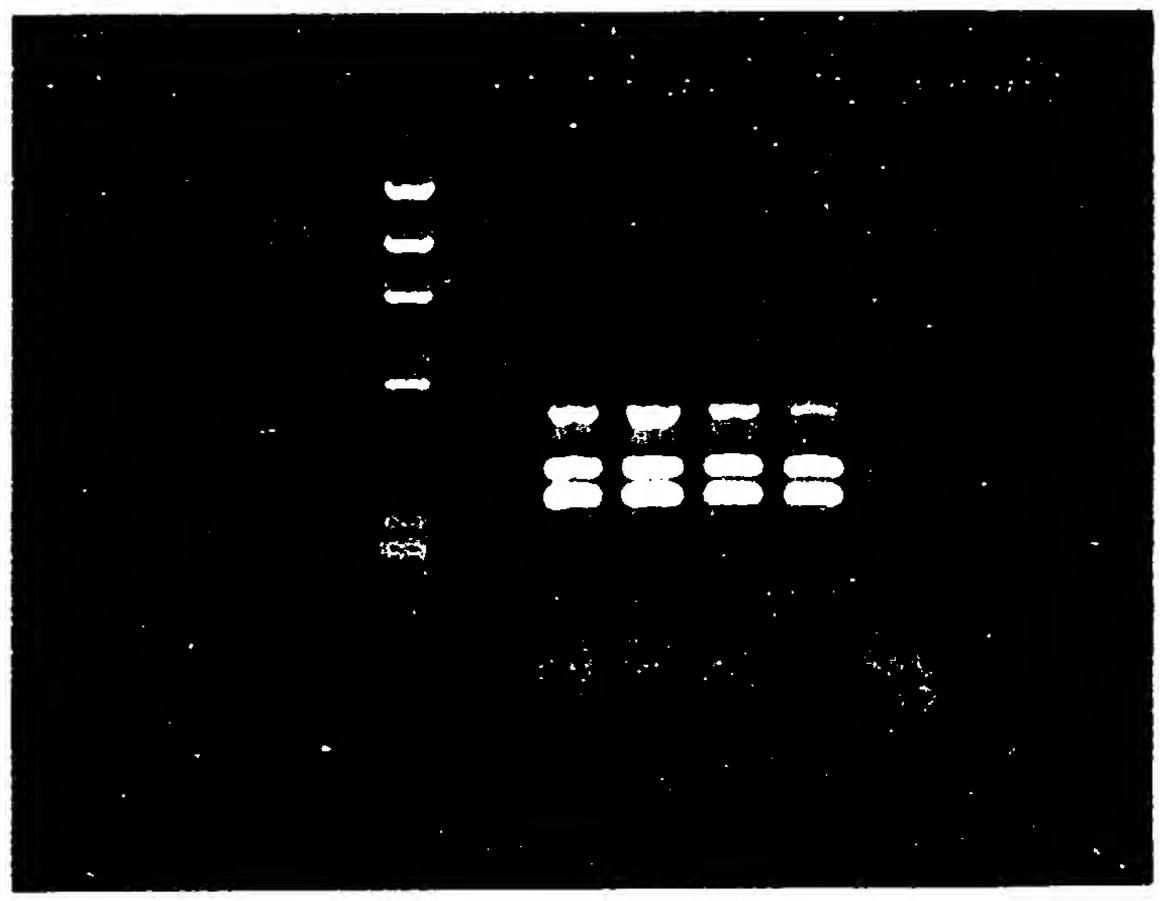


Joel Ravier

PCR #22

4/18/8

	1	2	3	4 1	5 2	6 3	7 4	8 5	9
1				776	776	776	776		
2	template			6	6	6	6		
3									
4	primers	221		2.2	2.2	2.2	4.4	2.2	
5		222		2.4	2.4	2.4	4.8	2.4	
6		251		1.95	1.95	1.0	1.0	1.95	
7		252		2.2	2.2	1.1	1.1	2.2	
8		276		2.2	2.2	1.1	1.1	2.2	
9		303		2.20	2.20	1.1	1.1	2.2	
10									
11	5x Taq			20	20	20	20	20	
12									
13	dNTP's			6	6	6	6	6	
14									
15	DMSO			10	10	10	10	10	
16									
17	H_2O			74.8	44.8	49.1	44.5	50.8	
18									
19	Add 2 λ Taq Pol (5x)								
20	Add 1 λ Taq Pol								
21	after 20 rds. to 2								
22									
23									
24									
25									
26									
27									
28	5 min 65°								
29									
30									
31									

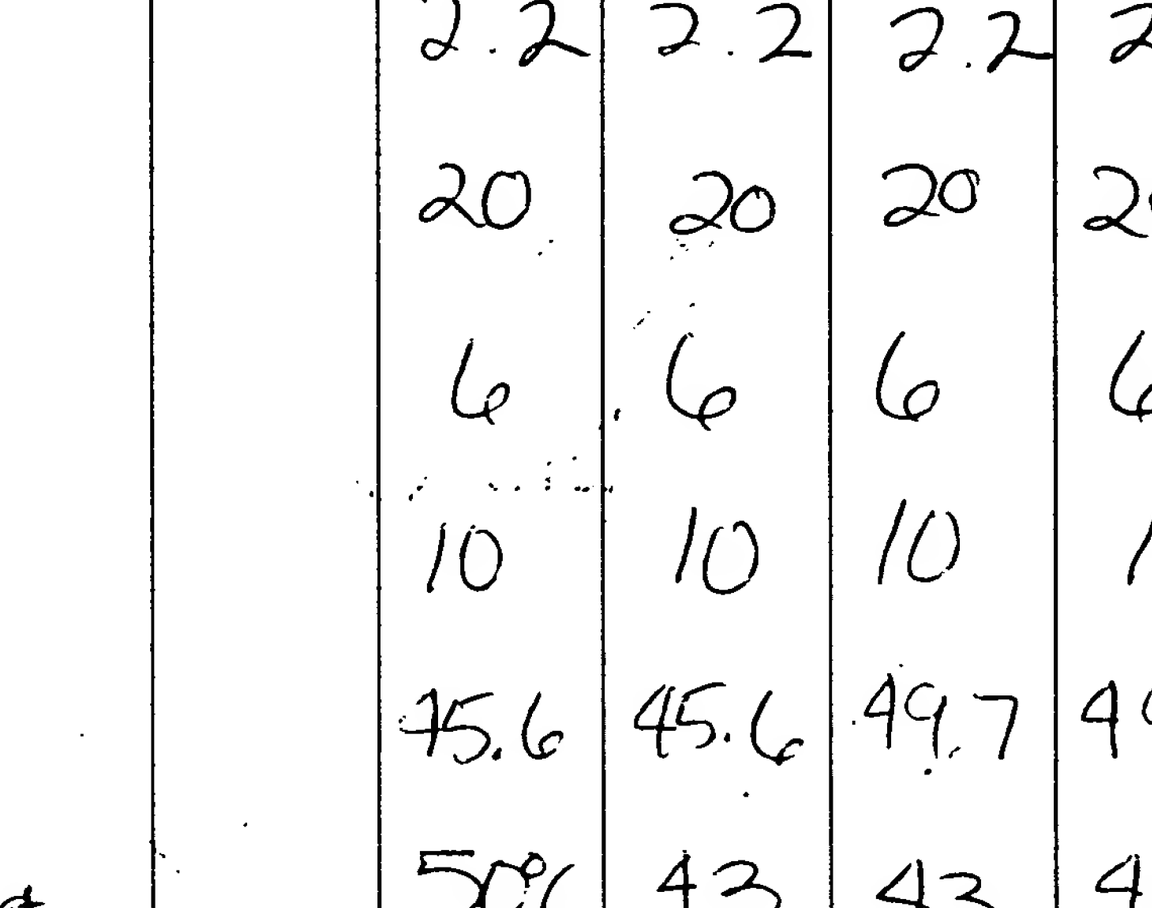


Joel Ranier

PCR #23

4/19/88

	1	2	3	4	5 1	6 2	7 3	8 4	9
1					776	776	776	776	
2	template				6	6	6	6	
3									
4	primers	221			1.9	1.9	1.9	—	
5		222			2.0	2.0	2.0	—	
6		251			1.95	1.95	—	1.95	
7		252			2.2	2.2	—	2.2	
8		276			2.2	2.2	2.2	2.2	
9		303			2.2	2.2	2.2	2.2	
10									
11	5x Taq				20	20	20	20	
12									
13	dNTP's				6	6	6	6	
14									
15	DMSO				10	10	10	10	
16									
17	H_2O				45.6	45.6	49.7	49.5	
18									
19	10 u enzyme at start				50C	43	43	43	
20	Extend 3 min.								
21	Extend 5 min at								
22	20 rounds								
23	Extend 7 min								
24	at end								
25									
26									
27									
28									
29									
30									
31									



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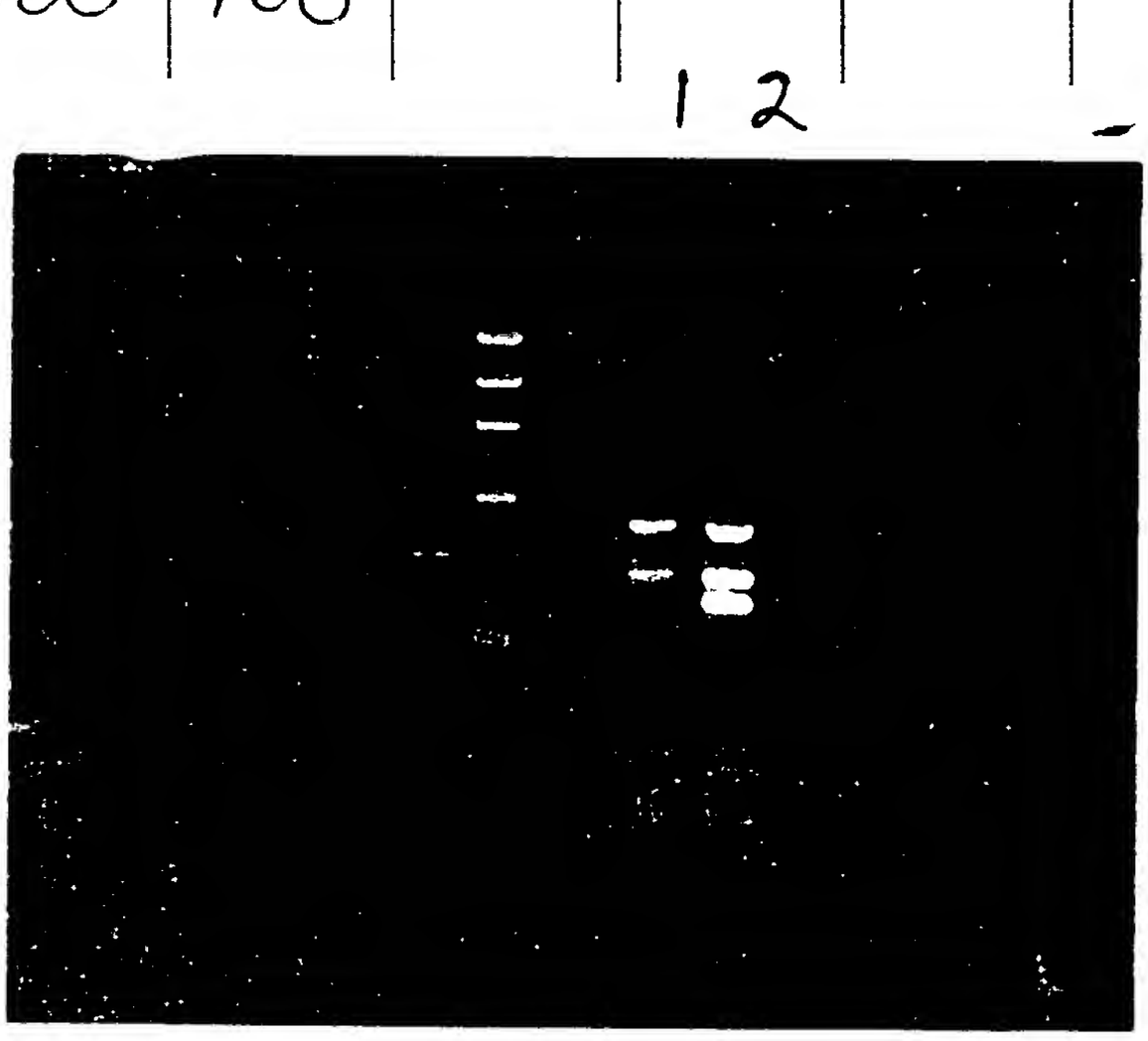
Joel Ranie

PCR #24 (to test stringency) 4/20/8

	1	2	3	4 1	5 2	6	7	8	9
1									
2	template			776	776				
3				6	6				
4									
5	primers	221		1.9	1.9				
6		222		20	2.0				
7		251		1.95	1.95				
8		252		2.2	2.2				
9		276		2.2	2.2				
10		303		2.2	2.2				
11									
12	5xTaq			20	20				
13									
14	dNTP's			6	6				
15									
16	DMSO			10	10				
17									
18	H_2O			45.6	45.6				
19				100	100				
20	Extend 35 min.								
21	10u Taq Pol.								
22									
23	1- 43°C anneal								
24	15sec								
25	2- 46°C anneal								
26	30 sec								
27									
28									
29									
30									
31									



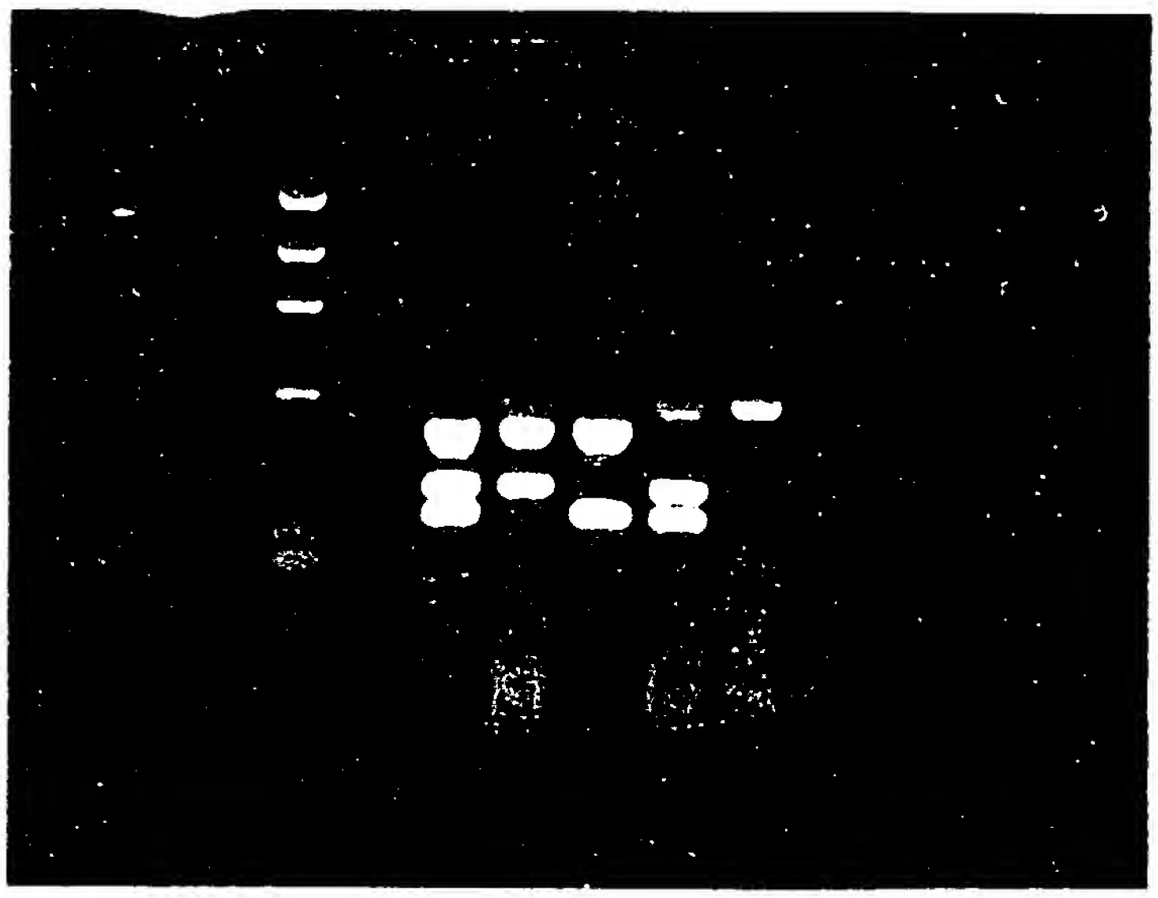
AMPAD EFFICIENCY LINE #: 22-206

PCR #25

4/20/8

	1	2	3	4 776	5 665	6 660	7 1011	8 JB	9 —
1				1	2	3	4	5	6
2	template			8.6	5.9	9.1	3.5	7.0	—
3									
4	primers		221	1.9	1.9	1.9	1.9	1.9	1.9
5			222	2.0	2.0	20	2.0	20	2.0
6			251	1.95	1.95	1.95	1.95	1.95	1.95
7			252	2.2	2.2	2.2	2.2	2.2	2.2
8			276	2.2	2.2	2.2	2.2	2.5	2.2
9			303	2.2	2.2	2.2	2.2	2.2	2.2
10									
11	5x Taq		20	→					→
12									
13	dNTP's		6	→					→
14									
15	DMSO		10	→					→
16									
17	H_2O			42.0	45.65	42.45	48.1	44.6	51.6
18									

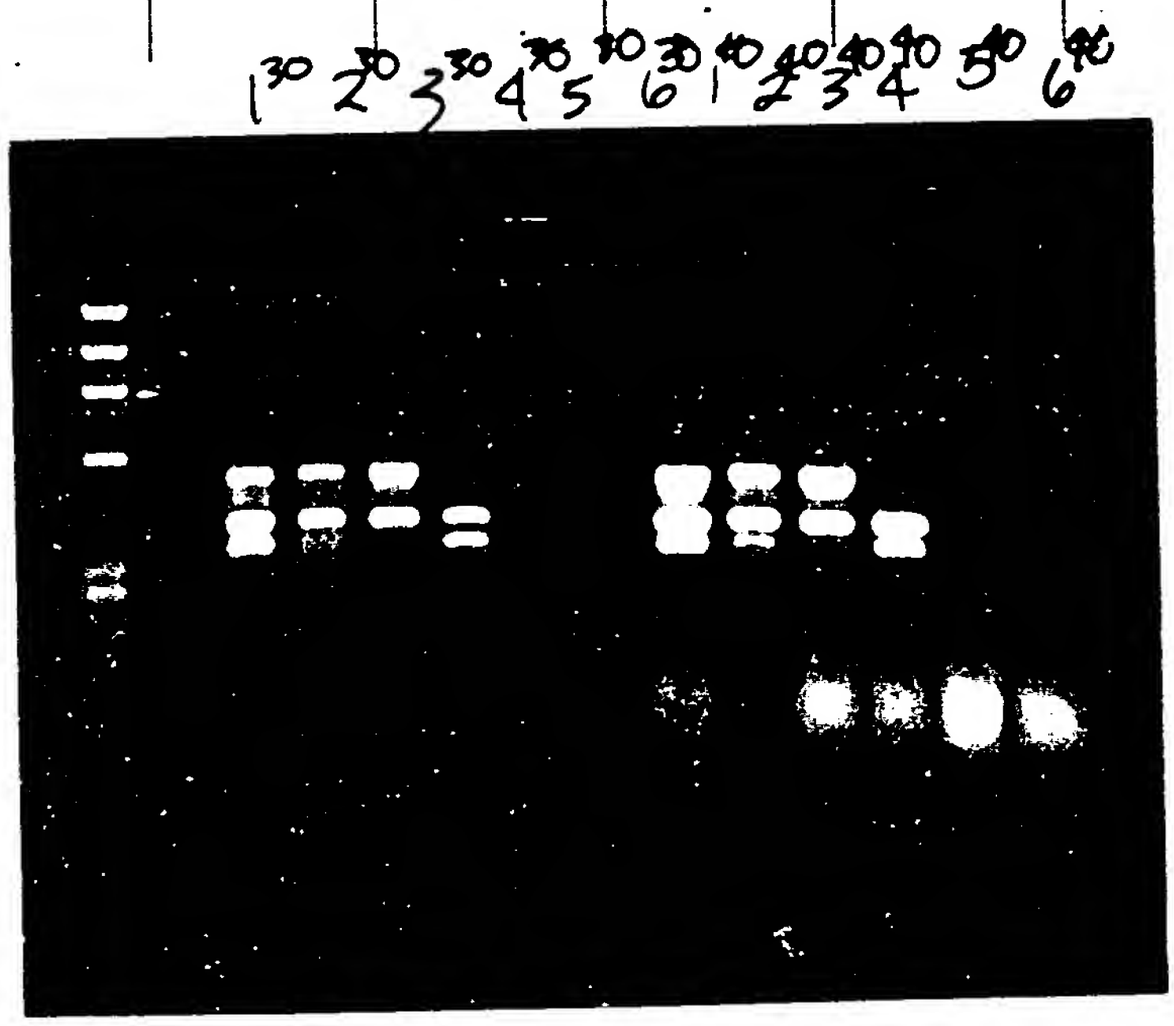
19 3.5 min extension
20 46°C anneal 30sec
21 7 min. denaturation
22 at beginning
23 7 min extension at end
24 2x (10u) Taq Pol. at
25 beginning



PCR # 26

4/25/8

	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9
1			774	774	665	1041	IB	←	
2	template		8.6	8.6	5.9	3.5	7.0	—	
3									
4	primers	221	1.9						
5		222	20						
6		251	1.7						
7		252	2.0						
8		276	2.2						
9		303	2.2						
10									
11	5x Taq	20							
12	dNTP's	6							
13	DMSO	10							
14	H_2O		43.4		+2.7	+5.1	+1.6	+8.6	
15	Mix			lenz.					
16	221	11.4							
17	222	12							
18	251	10.2							
19	252	12							
20	276	13.2							
21	303	13.2							
22	5x	120							
23	dNTP's	36							
24	DMSO	60							
25	H_2O	260.4							
26		Add 91.4							
27	47° 3 min. extension								
28									
29									
30									
31									



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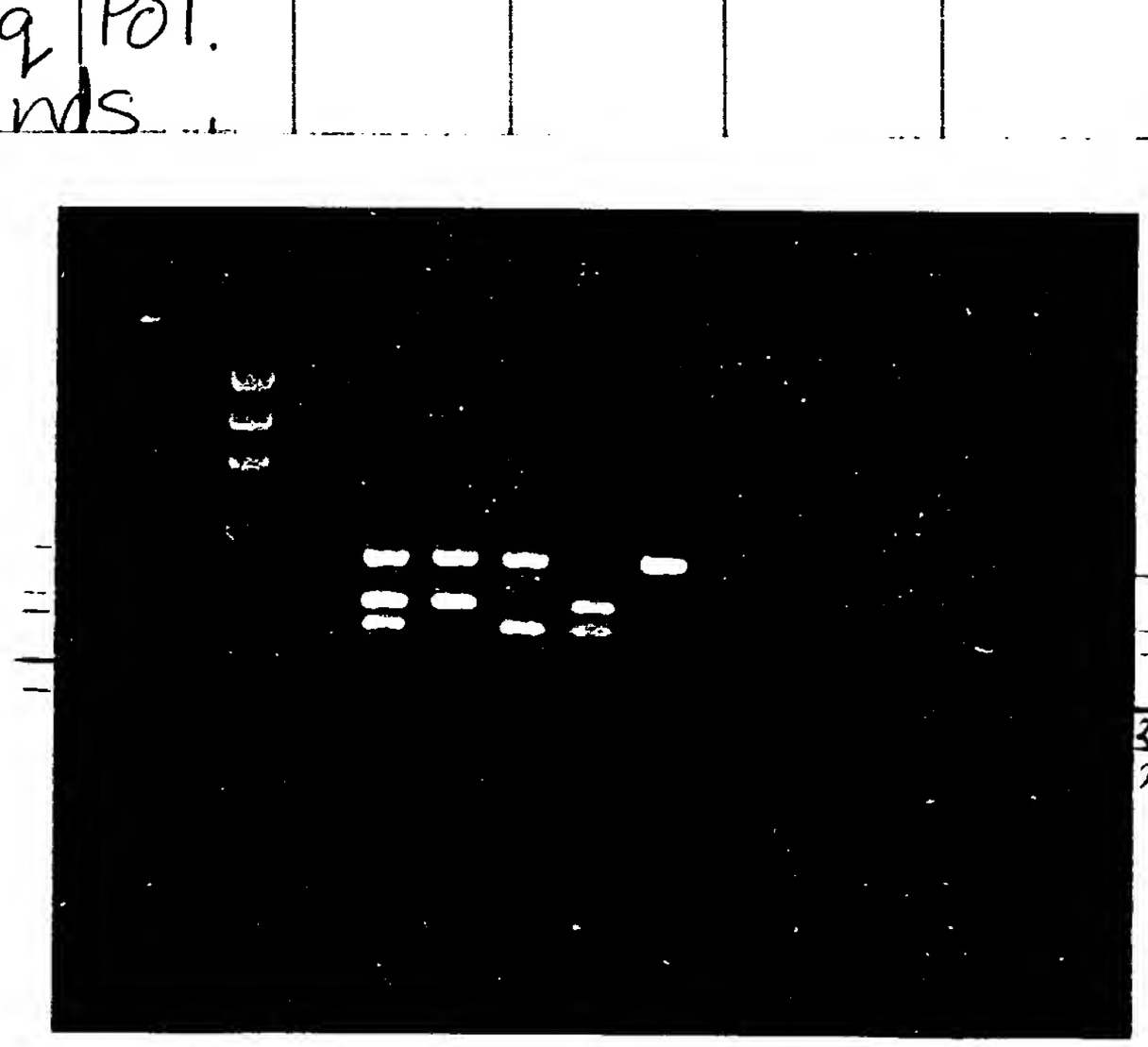
Joel Ranier

PCR #27 (All deletion combo's) 9/27/8

	1	2	3 1	4 2	5 3	6 4	7 5	8 6	9 7
1			5.56	5.9	8.7	3.5	5.70	7.0	
2	template		776	665	660	1011	691	JB	—
3			(90ng/λ)	(85ng/λ)	(57.5ng/λ)	(142.5ng/λ)	(87.5ng/λ)	(71ng/λ)	—
4	primers								
5	221	(46μM)	2.17						
6	222	(49μM)	2.0						
7	251	(59μM)	1.7						
8	252	(49μM)	2.0						
9	276	(51μM)	1.96						
10	303	(46μM)	2.17						
11									
12	5x Taq Buff		20						
13	DMSO		10						
14	dNTP's		6						
15			+3.14	+2.8	(43.3)	+5.2	+3	+1.7	+8.7
16	Mix	(7)							
17	221	15.2							
18	222	14							
19	251	11.9							
20	252	14							
21	276	13.70							
22	303	15.2							
23	5x	140							
24	DMSO	70							
25	dNTP's	42							
26	H_2O	303							
27	Add	91.3							
28									
29		5λ loaded							
30									
31									

3min extensions
2x Taq Pol.
30 rounds
47°C



368
201

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Joel Ranier

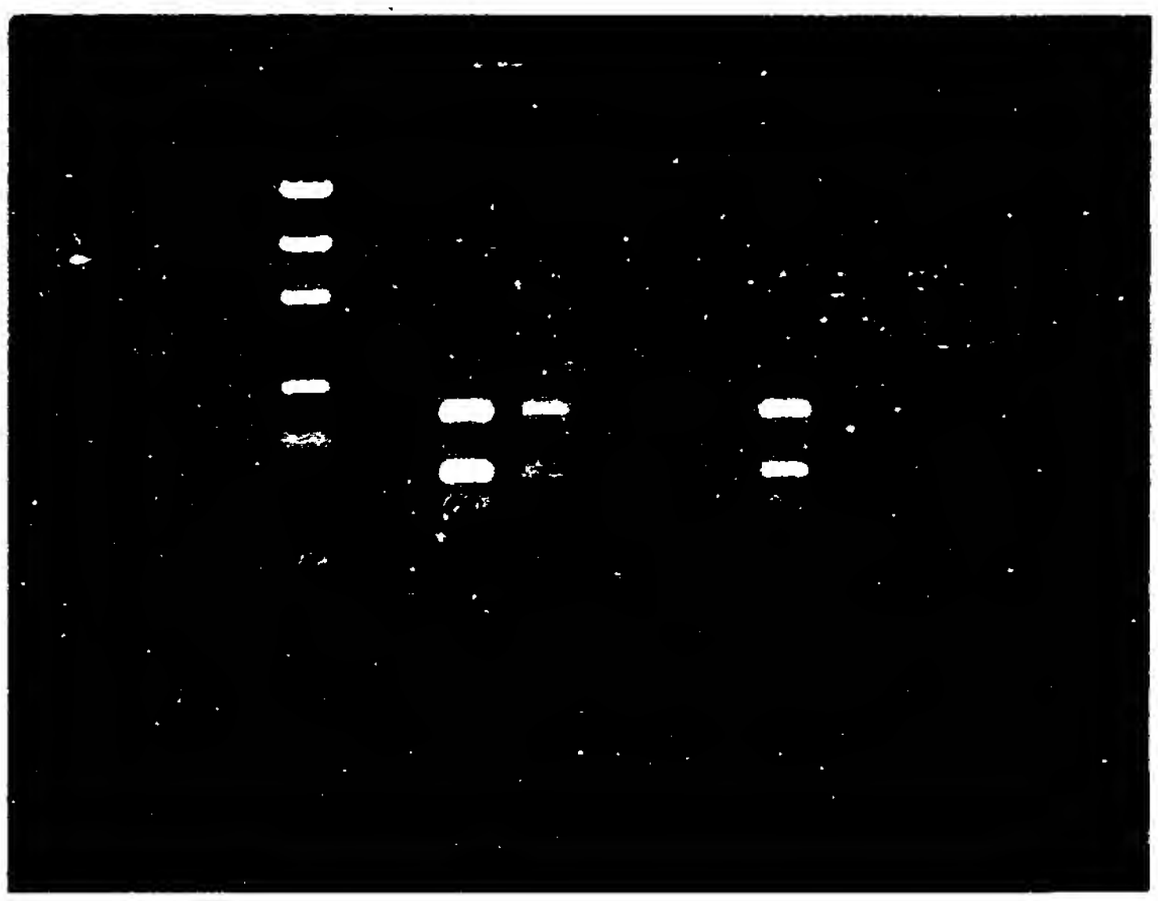
PCR (PNP's) #28

5/18/8

	1	2	3	4 776	B. Jonon	A. Jonon	Posey	Ruggiero	9 —
1				1	2	3	4	5	6
2	template			6	7.5λ	13.3λ	11.9λ	2λ	—
3									
4	primers	221		2.17					
5		222		2.0					
6		251		1.7					
7		252		2.0					
8		276		1.96					
9		303		2.17					
10									
11	5x Taq			20					
12	DMSO			10					
13	dNTP's			6					
14	H_2O			7.3	5.8	—	1.4	11.3	13.3
15									
16									

	Mix		X6	
17				
18	221	2.17	13.0	2
19	222	2.0	12.0	4
20	251	1.7	10.2	
21	252	2.0	12.0	
22	276	1.96	11.76	
23	303	2.17	13.0	
24	5x	20	120	
25	dNTP's	6	36	
26	H_2O	38.7	232.2	
27	DMSO	10	60	
28	Add		86.7	
29				
30				
31				

1 2 3 4 5



Dilutions for PND's

5/18/8

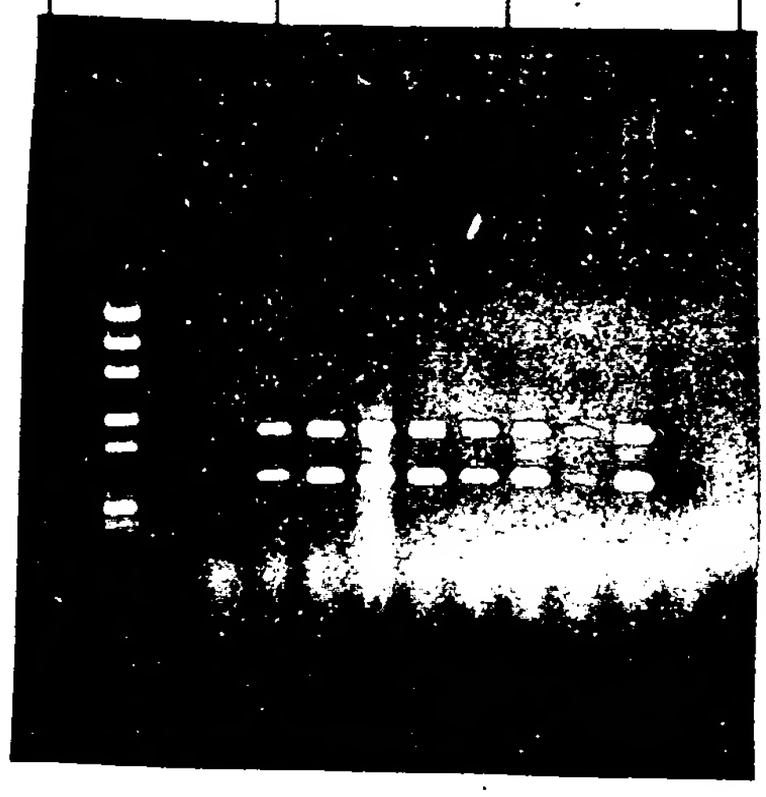
	1	2	3	4	5 DNA	6	7 H_2O	8 A_{260}	9 Conc.
1									
2	Russell	Jonon	(860ng/λ)		2.3		17.7	.016	67ng/λ
3	(Amnio)	Jonon	(500ng/λ)		4		16	.009	37.5ng/λ
4	776				8		92	.020	83.3ng/λ
5	Nicholas	Posey	(1.65ng/λ)		1.2		18.8	.185	
6	(Fetus)	Ruggiero	(260ng/λ)		7.7		12.3	.045	~~188~~ng/λ
7								0.01	42ng/λ
8								0.066	250ng/λ
9	Nick	Posey						0.058	240ng/λ
10									
11									
12									
13									
14									
15		Branch	3505	1000ng/λ	2		18	0.031	77.5ng/λ
16									
17	Lynn	Rousseau		585ng/λ	3.4		16.6	0.151	378ng/λ
18								0.022	155ng/λ
19	(Fetus)	Rousseau		100ng/λ	—		—		
20									
21	Mary	Richardsen		430ng/λ	4.7		15.3	0.151	378ng/λ
22								0.028	70ng/λ
23	Sharon	Watkins		74ng/λ	2.7		17.3	0.063	200ng/λ
24								0.069	172.5ng/λ
25	Alfred	Watkins		220ng/λ	9.1		10.9		
26									
27	(Fetus)	Watkins		470ng/λ	4.25		15.75	0.015	37.5ng/λ
28									
29		1504		700ng/λ	2.9		17.1	~~0.193~~	
30								0.199	500ng/λ
31								0.010	27.0ng/λ

Joel Ranier

PCR 29 (more PNPs)

5/18/18

	1	2	3	4	5	6 DNA	7 H_2O	8	9
1									
2	template	(1)	Posey (240ng/λ)			2.08λ	*	off. ♂ ×J1.1 → J-6 ♀	
3		(2)	Branche (77.5ng/λ)			6.5λ	13,5	aff ♂ 9-7	
4		(3)	L. Rousseau (55ng/λ)			9.1λ	10.9	carrier ♀	BNO
5		(4)	F. Rousseau (100ng/λ)			5λ	15	fetus - normal	
6		(5)	M. Richardson (70ng/λ)			7.15λ	12.85	carrier ♀	
7		(6)	S. Watkins (200ng/λ)			2.5λ	17.5	carrier ♀	
8		(7)	A. Watkins (172.5ng/λ)			2.9λ	17.1	off. male 9-7	BNO
9		(8)	F. Watkins (37.5ng/λ)			13.3λ	6.7	norm. fetus	
10		(9)	1504	(25ng/λ)		20λ	—	off male 9-7 BNO?	
11		(10)						neg. control	
12		~~(11)~~	×1	×8		* For (1)			
13	primers	~~251~~	1.7	13.6		221	2.17		
14		252	2.0	16.0		222	2.0		
15		276	1.96	15.7		251	1.7		
16		303	2.17	17.4		252	2.0		
17		5× Buff	20	160		276	1.96		
18		dNTP's	6	48		303	2.17		
19		DMSO	10	80		Buff	20		
20		~~H_2O~~	36.17	289.4		NaG's	6		
21			Add	80		DMSO	10		
22						H_2O	50		
23	2× enz.								
24	47°C anneal								
25									
26									
27									
28									
29									
30									
31									



6/2/88 DNA samples for PCR on DMD males

	DNA #	CONC.	NAME		ORL #
* 1st Priority Group	346 - ①	.997	George Derry, Jr. -	47-46	22B
	472 - ②	.824	Thomas Davis		70B
	3934 - ③	.599	Shane Worth		521
	3955 ④	.757	Alan Cox		522
	3920 ⑤	.858	Douglas Hazelton -	47-46	523
	3944 ⑥	1.054	Billy Buchanan		524
	3948 ⑦	.938	Andrew Roybal		531
2nd priority Group	3929 ⑧	.712	Keith young III -	47-46	505
	~~346~~		~~robert barnes Jr~~		~~473~~
	3940 ⑨	.220	SCOTT miller ?		484
	3860 ⑩	1.02	Donald Capello		513
	3880 ⑪	.510	Wayne NOON		514
	3895 ⑫	.606	David Van Zandt		519
	3950 ⑬	.809	Matthew Stone		520

Joel Ramler

Dilutions for Diagnostic PCR

6/2/18

	1 DNA #	2 Conc.	3 For 500ng	4 DNA (µl)	5 H_2O (µl)	6	A_{260}	8 Conc	9 Add H_2O	
1										
2	346	1.0µg/λ	5.23	2.5	17.5		0.181	754ng/λ	+100.6	95.8ng/
3							0.023			
4	472	824ng/λ	1.67	3.0	17.0		0.127	529ng/λ	+66.1	300ng/
5							0.072			
6	3934	600"	8.0	4.2	15.8		0.044	183ng/λ	+9.3	625ng/
7							0.015			
8	3955	757"	7.5	3.3	16.7		0.049	209ng/λ	+12.6	66.7ng/
9							0.016			
10	3920	858"	3.88	2.9	17.1		0.031	129ng/λ		1
11										
12	3944	1.0 µg/λ	5.71	2.5	17.5		0.045	187.5ng/λ	+10.00	87.5ng/
13							0.021			
14	3948	940ng/λ	3.25	2.65	17.35		0.037	154ng/λ		
15										
16	3929	712ng/λ	3.14	3.5	16.5		0.038	158ng/λ		
17										
18	3940	220"	7.5	11.36	8.64		0.131	546ng/λ	+67.3	66.7ng/
19							0.016			
20	3860	1.0µg/λ	8.0	2.5	17.5		0.040	167ng/λ	+6.72	625ng/
21							0.015			
22	3880	510ng/λ	10.0	4.9	15.1		0.012	50ng/λ		
23										
24	3895	606"	4.27	4.13	15.87		0.028	117ng/λ		
25										
26	3950	809	3.74	3.1	16.9		0.032	133ng/λ		

27	Oligo		A_{260}	Conc.
28	222-1	(27-mer)	.537	150.7µM
29	221	(27-mer)	0.48	136µM
30	222-2	(27-mer)	0.73	205µM
31				

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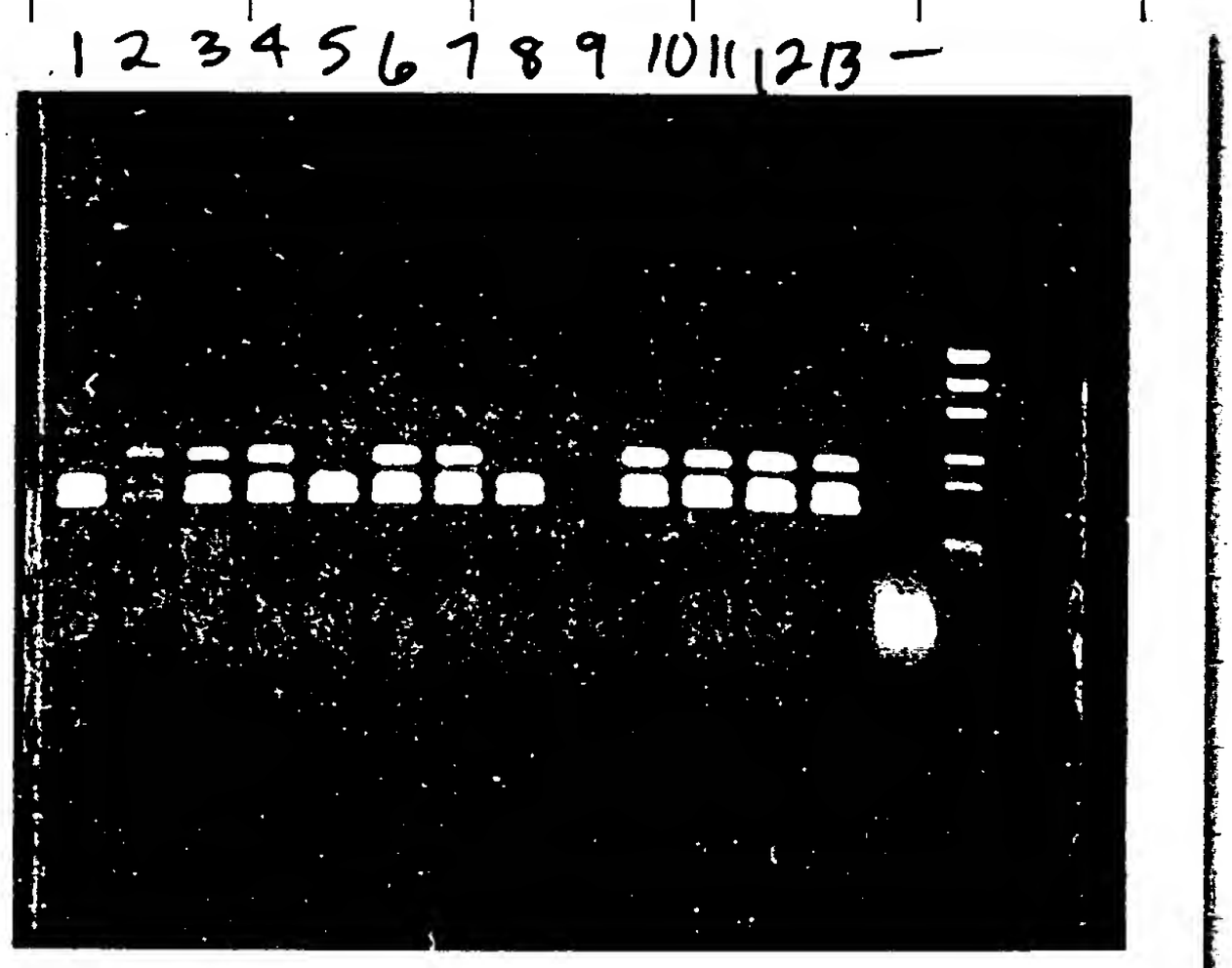
Joel Parker

PCR 30 (diagnostic) 6/4/8

	1	2	3946	3972	3934	3955	3920	3944	3948	3929	3940	3860	3880	3895	3950	—
1			1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	template		6.23	1.67	8.0	7.5	3.88	5.71	3.25	3.16	7.5	8.0	10.0	9.27	3.76	—
3			√	√	√	√	√	√	√	√	√	√	√	√	√	
4	H_2O		4.17	8.33	2.0	2.5	6.12	4.29	6.75	6.84	2.5	2.0	—	5.73	6.24	10.0
5																
6	primer	221	(136 μM)													
7		222	(151 μM)													
8		251	(223 μM)													
9		252	(208 μM)													
10		276	(106 μM)													
11		303	(122)													
12																
13	Mix	1x	13x													
14	5x Buff	20	260													
15	DMSO	10	130													
16	dNTPs	6	78 (5)													
17	221	0.74	9.56													
18	222	0.66	8.61													
19	251	0.45	5.83													
20	252	0.48	6.25													
21	276	0.94	12.26													
22	303	0.82	10.66													
23	H_2O	49.9	648.83													
24			Add 90λ													
25																
26	2x Taq Pol.															
27	47°C															
28																
29																
30																
31																



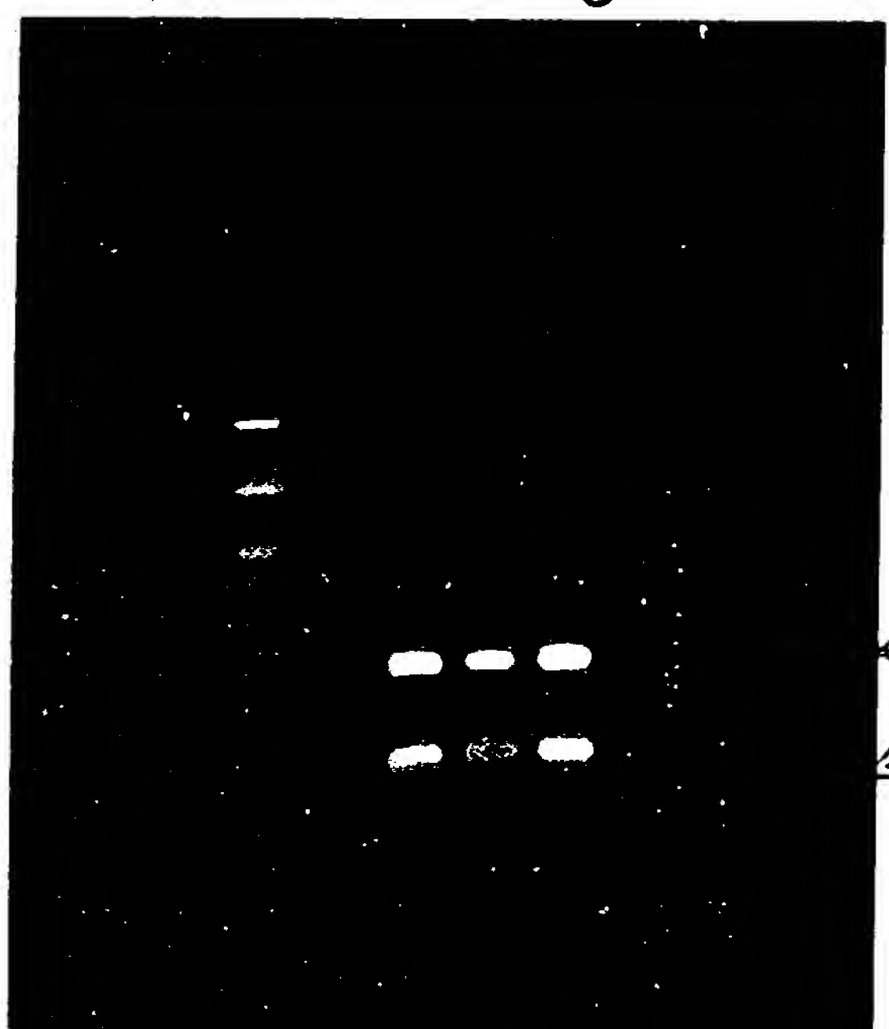
10λ

Joel Rainis

PCR 31 (diagnostic)

6/9/8

	1	2	3 1	4 2	5 3	6	7	8	9
1	H_2O	5	38.47 5λ	39.61 10λ	39.68 3λ				
2	template	2	5		7				
3		Mix		1x	4x				
4	primers	221	(13 μM)	.735	2.94				
5		222	(150 μM)	.66	2.65				
6		251		1.7	6.8				
7		252		2.0	8.0				
8		303	(122 μM)	.82	3.28				
9		276	(51 μM)	1.96	7.84				
10	5x Buff	90							
11	DMSO	40							
12	dNTP's	24							
13	H_2O	184.5							
14									
15	Add	90λ							
16									
17									
18									
19									
20	* Check to see if								
21	a primer (221, 222)								
22	was left out								
23	see 6/15/8 2,3,4								
24									
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE™ 22-206 AMPAD

Gord Karlen

Dilution of DNA (affected ♂s) 31

6/9/8

	1	2	3	4 DNA	5	6 H_2O	7	8 A260	9 Conc
1									
2	3847	782 ng/λ		3.2		16.8		.029	100 ng/λ
3	Douglas Eastman								
4									
5	3961	105 ng/λ		—		—		0.109	
6	Norman Hillman							0.012	50 ng/λ
7									
8	3968	907 ng/λ		2.7		17.3		.040	167 ng/λ
9	Christopher Lipscomb								
10									
11	3 → 250λ								
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Gold Rans

PCR 31b (to test to confirm PCR 31 primers 395, 396) 6/14/18

	1	2	3	4 776	5	6 5847	7 3961	8 3968	9
1				X		2	3	4	
2	template			6		5	10	3	
3									
4	primers	395	( )		221	2.17	2.17	2.17	
5		396	( )		222	2.0	2.0	2.0	
6									
7	5x Buff			20		20	20	20	
8	DMSO			10		10	10	10	
9	dNTP's			6		6	6	6	
10									
11	H_2O					54.83	49.83	56.83	
12									
13						2.0 min extension			
14	2u Taq					47°C			
15	30 rounds								
16	47°C								
17									
18	Dilution of 221								
19		222-1							
20		395							
21		396							
22		303 (524)				Photo 6/15/18			
23		252 (51.2)				234			
24									
25									
26									
27									
28									
29									
30									
31									

AMPAD EFFICIENCY LINE™ 22-206

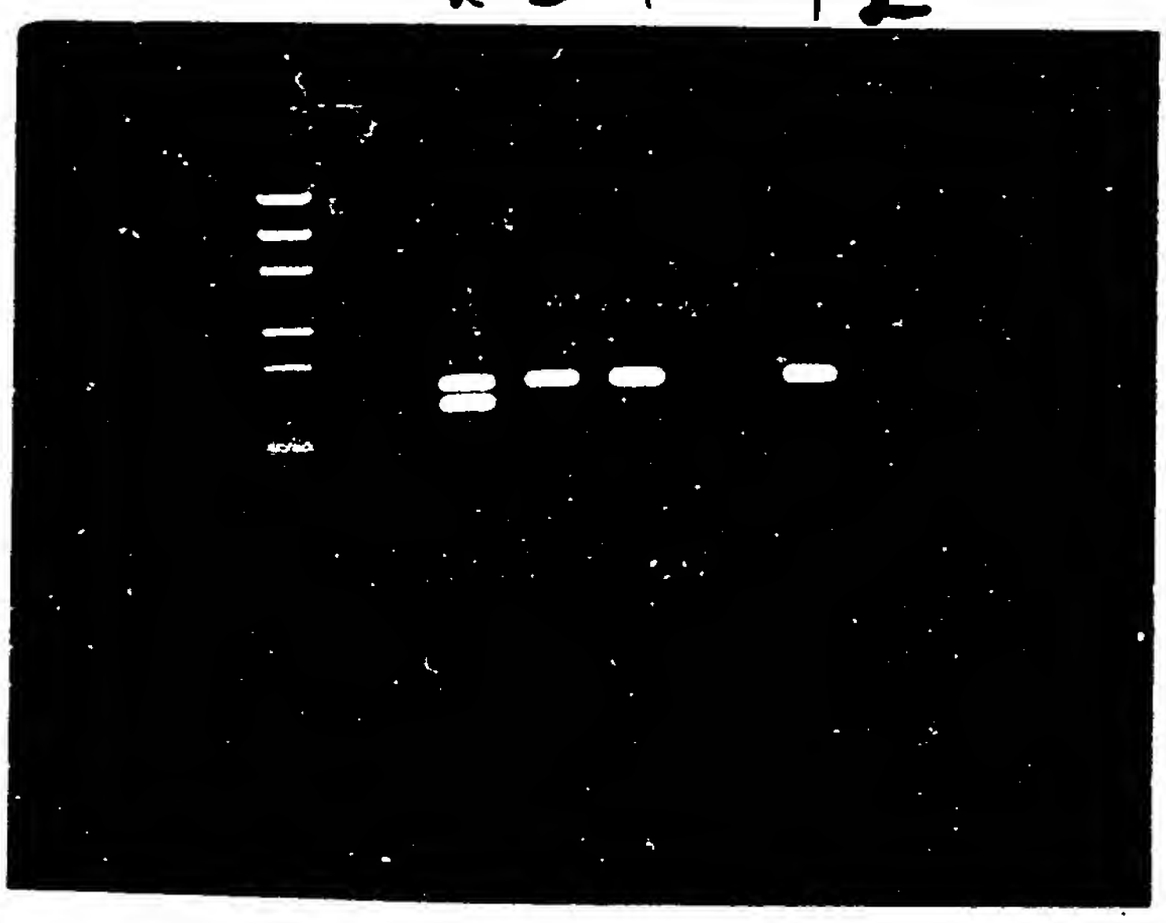
Joel Ranier

PCR 33 (to test 395, 396) 6/15/8

	1	2	3	4	5 776	6 776	7 776	8
1					1	2	X	
2	Template				6	6	6	—
3	primers	221	(46)		2.17	—	2.17	
4		222	(49)		2.0	—	2.0	
5		251	(59)		~~1.7~~	—	1.7	
6		252	(51.2)		~~1.95~~	—	1.95	
7		376	(51 MM)		~~1.96~~	—	1.96	
8		303	(52.4)		~~1.9+~~	—	1.91	
9		395	(56.3)		—	1.78	1.78	
10		396	(91.2)		—	1.1	1.1	
11								
12								
13	~~5~~ 2x Buff				20	20		
14	DMSO				10	10		
15	dNTP's				6	6		
16	H_2O				46.3	55.1	49.4	
17								
18						47°C		
19						1 min extension		

Confirmation of 31

2 3 4 1 2 *



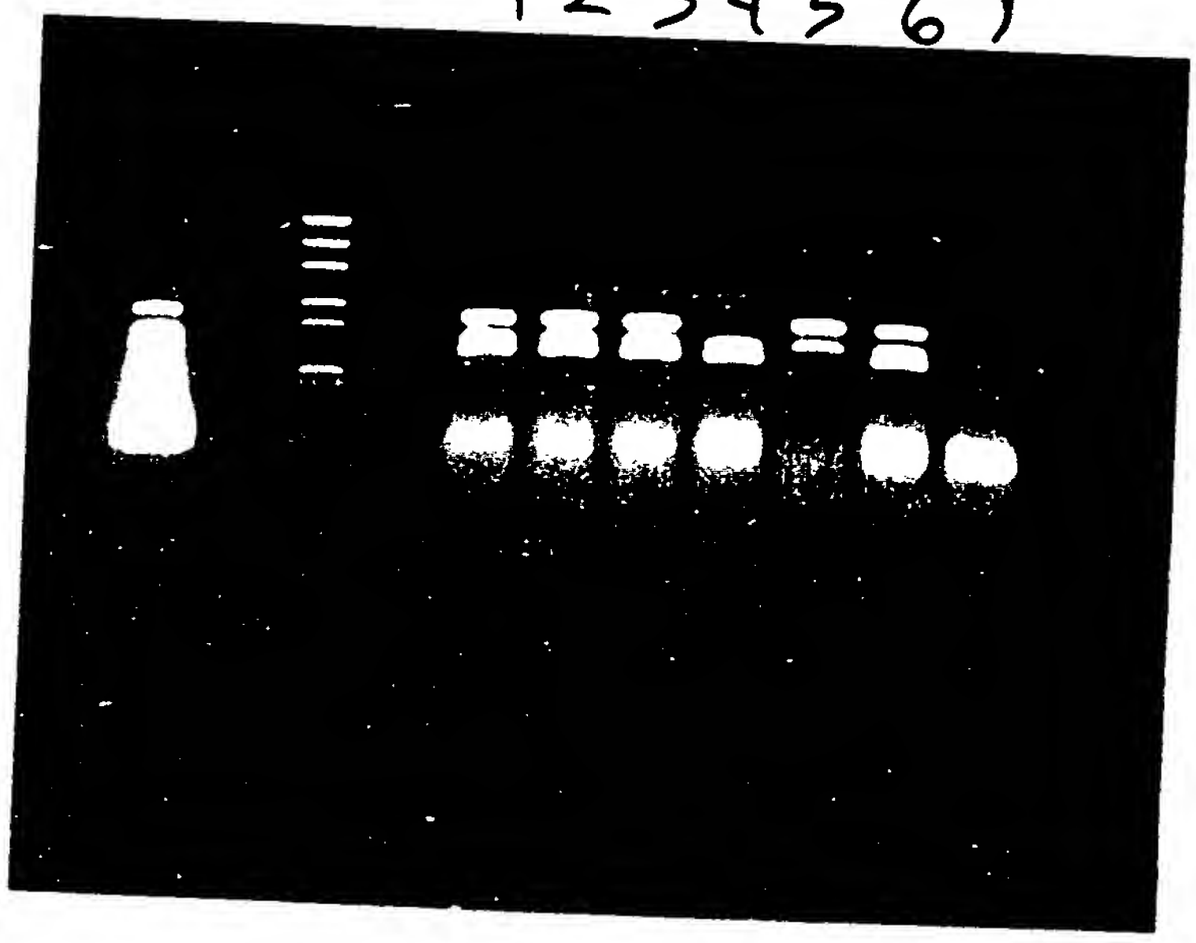
pos. con. →

* R.G. cut purified digests out of gel & switched

Joel Ravi

PCR 34 (1) PND's and others 6/21/8

	1	2	3288	3989	2945	3987	748	3986	9 —
1			1	2	3	4	5	6	7
2	template		7.1	3.5	10.9	2.22	8.0	3.0	—
3	H_2O		3.8	7.4	—	8.7	2.9	7.9	10.9
4									
5	Mix	1x	7x						
6	221	0.14	5.15						
7	222	0.66	4.6						
8	251	0.45	3.14						
9	252	0.48	3.37						
10	276	0.61	4.3						
11	303	3.6	25.1						
12	5x Buff	20	140						
13	DMSO	10	70						
14	dNTP's	6	42						
15	H_2O	46.56	325.92						
16		Add	89.1						
17									
18	2λ Taq Pol.								
19	3 min ext.								
20	47°C								
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



AMPAD EFFICIENCY LINE #: 22-206

	1	2	3	4	5	6	7	8 A_{260}	9 Conc.
1	3988								
2	ERIC DAVIS			698ng/λ		3.6	16.4	.158	
3								.017	70.8ng/λ
4	3989								
5	ROBERT SURET, JR.			1700ng/λ		1.80	18.8	039	142ng/λ
6									
7	2995								
8	DANIEL WEBSTER			634ng/λ		3.9	16.1	.110	
9								.011	46ng/λ
10	3987								
11	JOSEPH TORRANO			598ng/λ		4.2	15.8	.009	~~10.7ng/λ~~
12								.054	225ng/λ
13	748 (DRL)								
14	BRIAN BLY			645ng/λ		3.9	16.1	.336	
15								.015	62.5ng/λ
16	3986								
17	MORRIS AMNIO			307ng/λ		8.0	5.0	.178	
18								.090	166.7ng/λ
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ravier

PCR Automation Trials

6/22/18

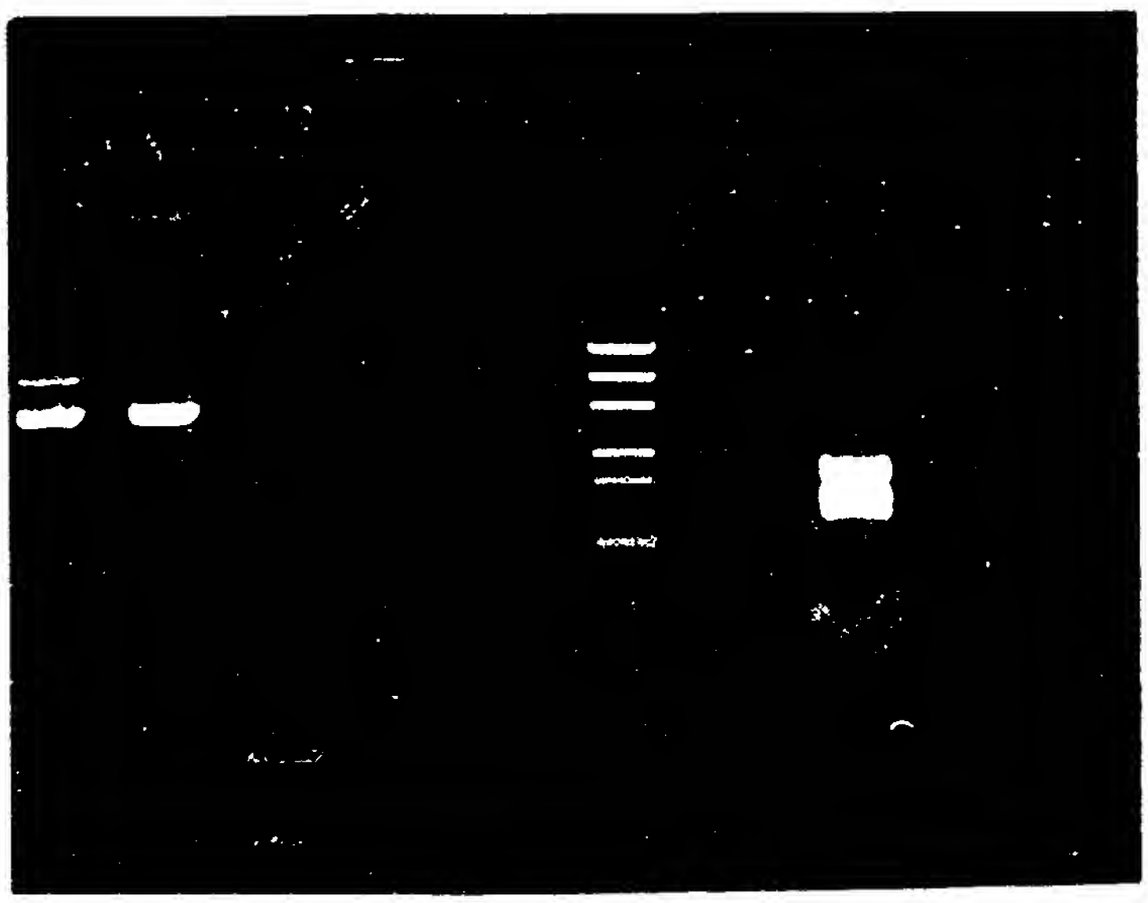
6/20/18

① No initial 7min. deraturation
35 cycles
1.5 min denat.
1.0 min anneal 47°C
3.15 min extension 65°C
7 min extension at end.
(oil overlay) 50λ

② 7 min denat.
35 cycles
1.5 min denat. 94°C
45 sec anneal 47°C
3.15 ~~sec~~ min extension 65°C
7 min. extension at end.
(oil 50λ)

(Picture see PCR 34 6/21/18)

PCR 2



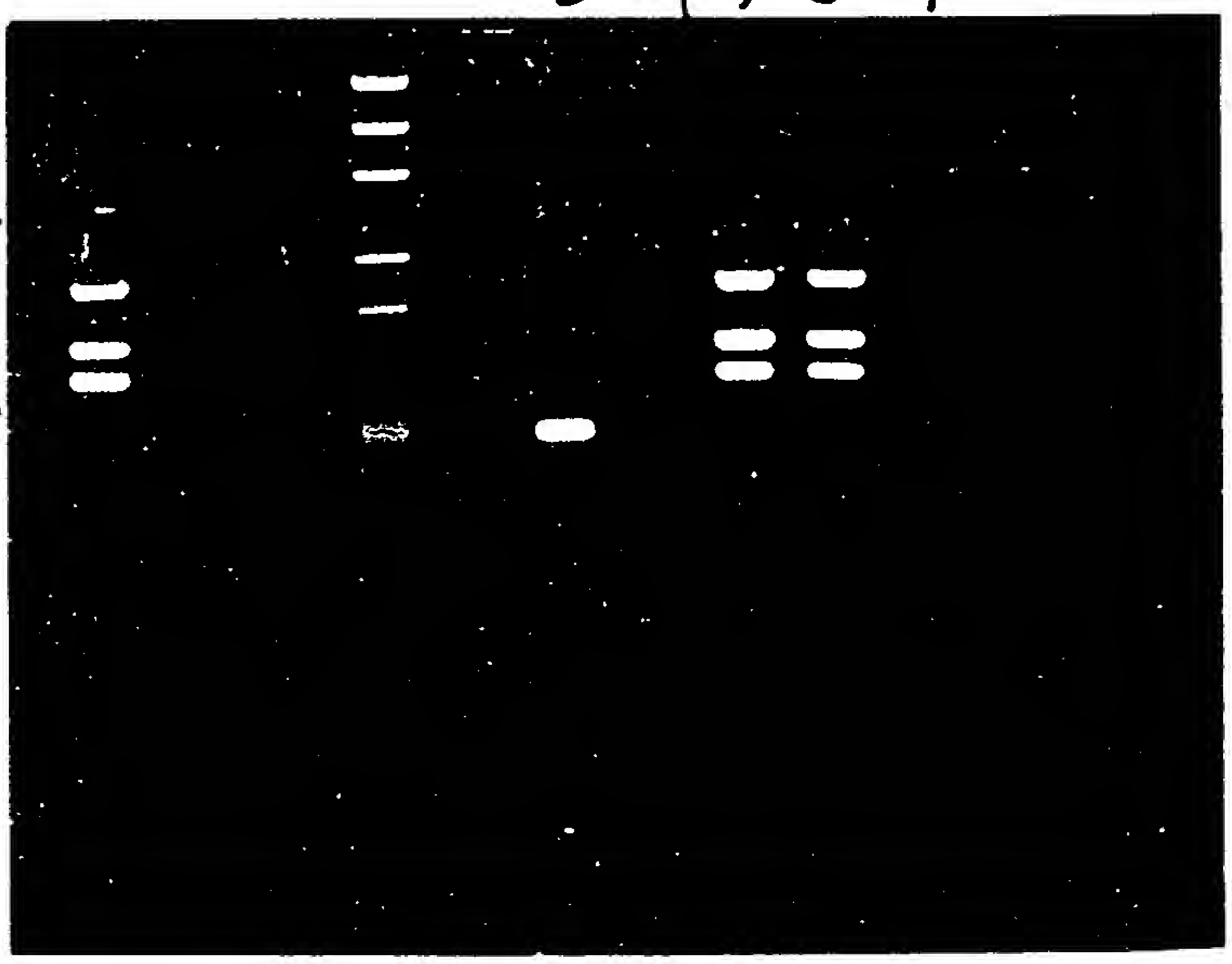
Joel Ramer

PCR 35 (to test new oligos [395, 396] + diagnostic cases) 6/23/98

1	2 (O/N 6/22)	X 1	X 2	3	4	5	6	7
		776	776	776	776	#6	#4012	—
Template		6	6	6	6	1.8	3.16	—
primers	221	0.14	2.17			2.17	2.17	2.17
	222	0.66	2.0			2.0	2.0	2.0
	251	0.45	1.7			1.7	1.7	1.7
	252	1.95	1.95			1.95	1.96	1.95
	276	1.00	1.96			1.96	1.96	1.96
(27.9)	303	3.96	3.6			3.6	3.6	3.6
(123)	395?√	3.60	.81	0.81		—	—	—
(91.2)	396	3.8	1.1	1.1		—	—	—
(91.2)	416	—	1.1		1.1	—	—	—
(61.8)	417	—	1.62		1.67	—	—	—
5x Taq		20						
DMSO		10						
dNTP's		6						
H_2O		41.99	40.0	56.1	55.3	48.8	47.5	50.6

ON PCR Machine
1:30 denat.
45s anneal *51°C
3:30 extend

3 4 5 6 7



~~||||~~ ~~||||~~ ~~||||~~ ~~||||~~

AMPAD · EFFICIENCY LINE® 22-206

	1	2	3	4	5	6 DNA	7 H_2O	8 A_{260}	9 conc
1	① Anthony Barrer			DRL #6		4.35	15.65	.088	283 ng/λ
2	575 ng/λ								
3									
4	② Alan Sparkman		4012			4.96	15.04	.078	158 ng/λ
5	504 ng/λ								
6									
7	416-1							0.313	91.2 μM
8									
9	417-1							0.212	61.8 μM
10									
11	303/295							0.406	123 μM
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

O.D.'s of Oligo's 395, 396

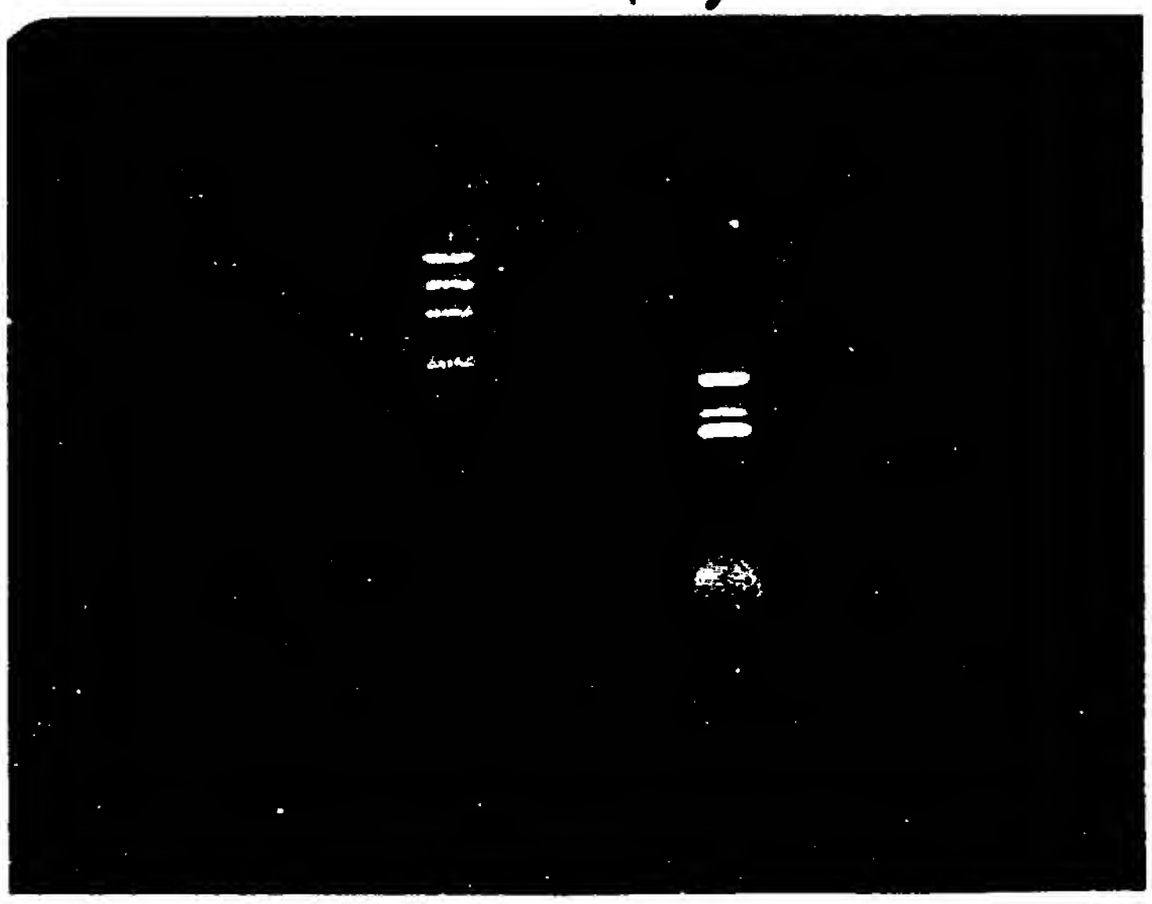
	1	2	3	4	5	6	7	8	9
1				A_{260}		Conc			
2									
3	395-1			.186		56.3 μM			
4	395-2			.227		68.8 μM			
5	396			.301		91.2 μM			
6	303			.092		27.9 μM			
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Ramirez

First Attempt [916, 417 (420, 421)]

6/27/18

	1	2	3	4	5	6	7	8	9
1				6λ					
2	template			77.4					
3			dil (27.9)						
4	primers	416	(110 μM)	3.6					
5		417	(8 μM)						
6			dil (17.2)	5.8					
7									
8									
9									
10									
11	H2O			48.6					
12									
13									
14									
15									
16									
17	2' extension								
18	37°C anneal								
19	1λ Taq								
20									
21	(2) 3 sets + 395								
22	396 on								
23	Thermocycler								
24									
25	* Incorrect								
26	primer conc.								
27									
28									
29									
30									
31									

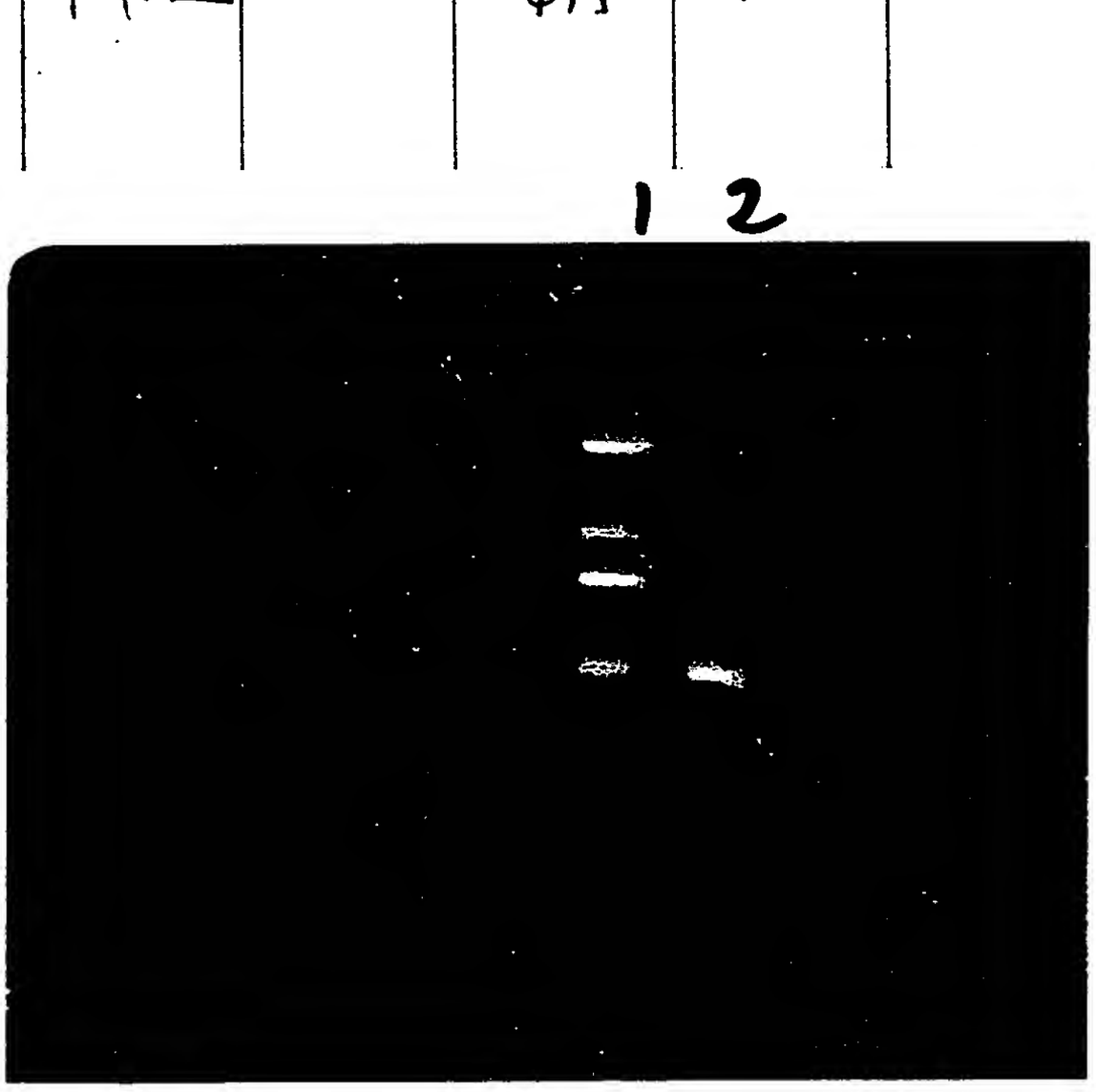


Joel Ranier

PCR 36 (on thermocycler) 6/28/8

	1	2	3	4 1	5	6 2	7	8	9
1				776		776			
2	template			4		4			
3									
4	primers	221		0.75		—			
5		222		0.65		—			
6		251		0.5		—			
7		252		2.0		—			
8		276		1.0		—			
9		303		2.0		—			
10		395		0.80		0.8			
11		396		1.1		1.1			
12									
13	5x Buff			20		20			
14	DMSO			10		10			
15	dNTP's			6		6			
16									
17	H_2O			49.2		56.1			
18									
19									
20									
21	denat. 1:30								
22	anneal 45	, 51°C							
23	ext. 3:30								
24	2x Taq Pol								
25									
26									
27									
28									
29									
30									
31									

1 2



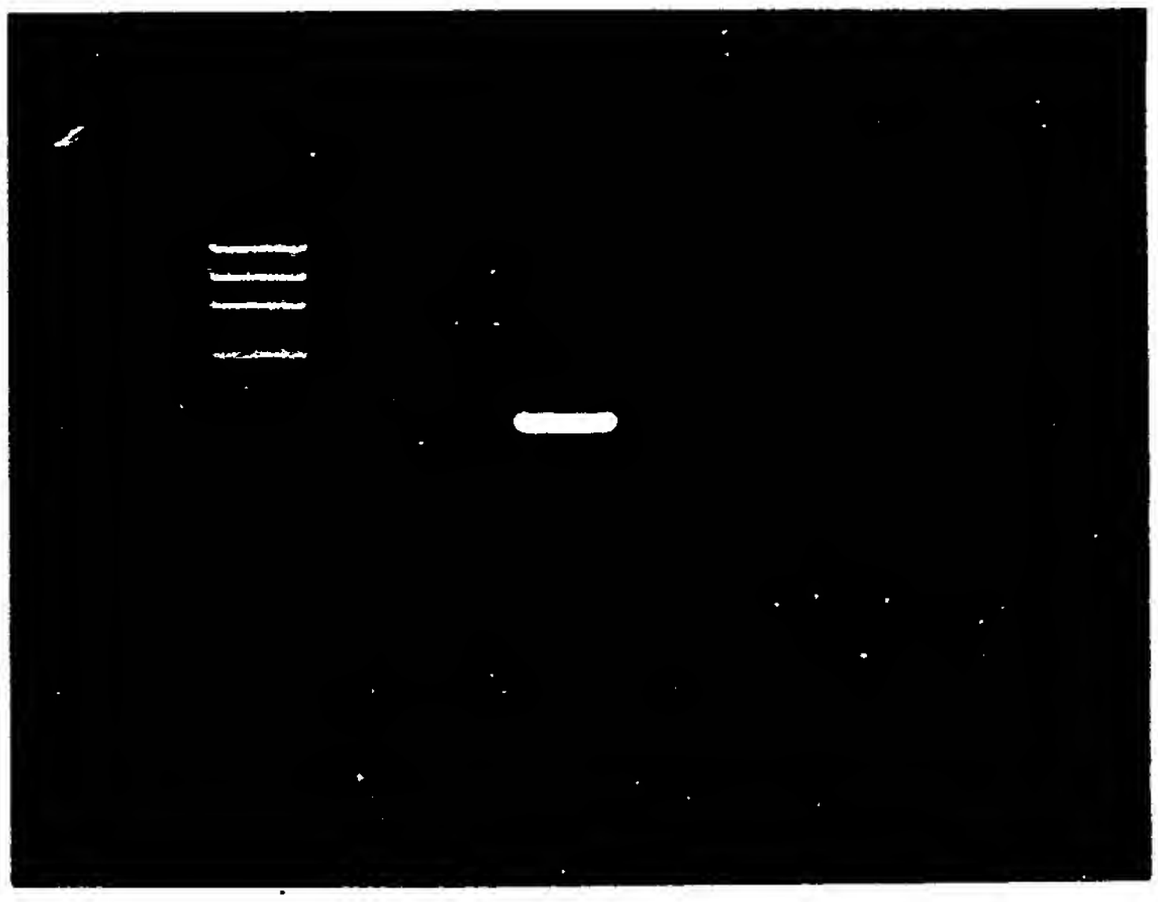
Dilutions of 416, 417, N. CTL - DNA 6/28/8

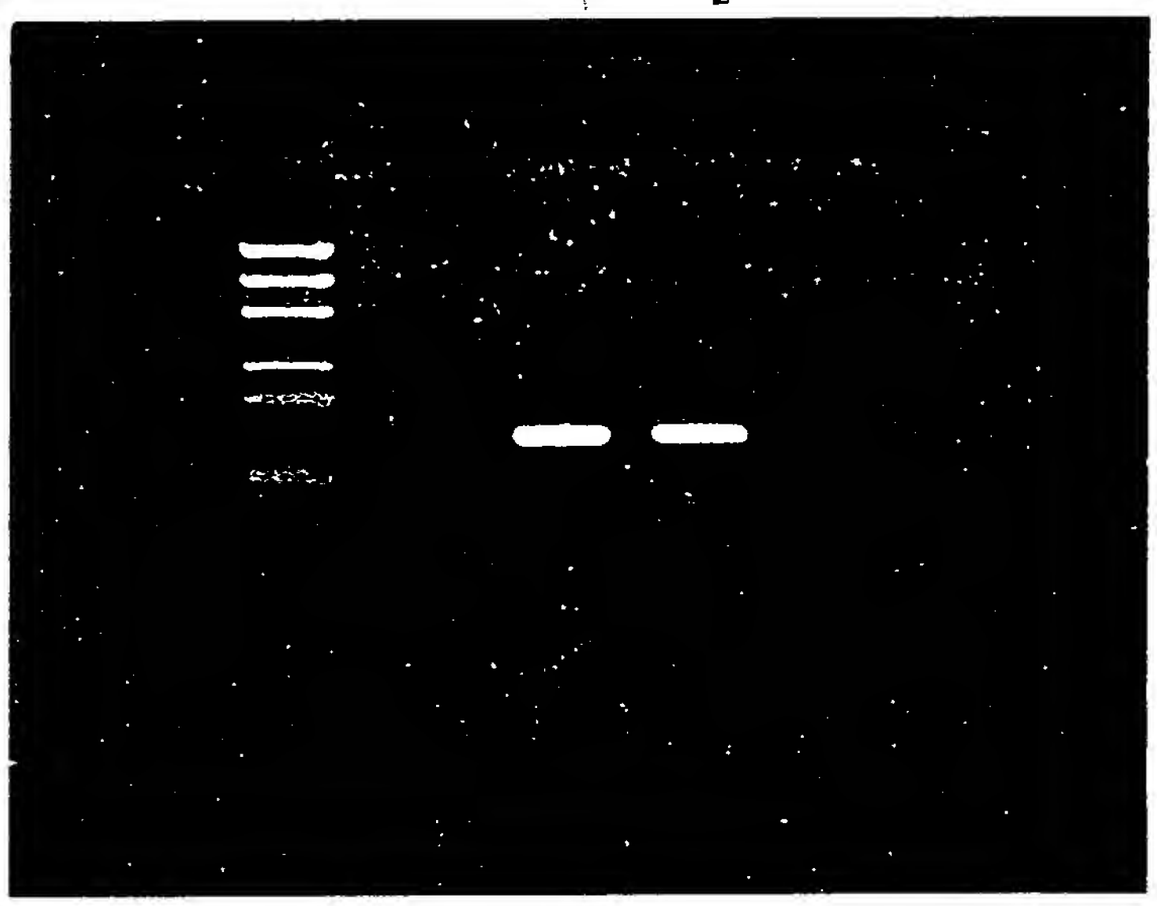
	1	2	3	4 H_2O	5	6 DNA	7	8 A_{260}	9 Conc.
1									
2	4162			~~39.3~~		~~10.7~~		0.401	116.8 μM
3									
4	417-2			~~25~~		~~25.1~~		0.171	49.8 μM
5									
6	416-2dil		(116.8)	39.3		10.7		0.082	23.9 μM
7									
8	417-2dil		(49.8)	25		25		0.092	26.8 μM
9									
10	416 2nd dil.		(416 stock 91.2)	36.3		13.7		0.09	26.2 μM
11									
12	417 2nd dil.		(416 stock 61.8)	30		20		0.077	22.4 μM
13									
14	N.CTL.		(1.16)	44.6		5.4		0.022	91.6 ng/λ
15									
16	257	(223)		46.4		5.6		0.099	30 ng/λ
17									
18	276	(163)		52.3		7.7		0.096	29 ng/λ
19									
20									
21	416	1:500							
22	417	1:200							
23									
24									
25									
26									
27									
28									
29									
30									
31									

Joel Rav

PCR 37 [to test 420 421 (416, 417)] 6/29/8

	1	2	3	4 1	5 2	6	7	8	9
1				N. CTL.	N.CTL				
2	template (91.6)			5.46	5.46				
3									
4	primers	251	(30)	3.33	3.33				
5		252	(51.2)	1.95	1.95				(test 2)
6		416-2dil.	(23.9)	4.2	4.2		1		7/5 4°C
7		417-2dil.	(26.8)	3.7					
8		416-2dil.	(23.9)	—					
9		416-2dil	(26.2)	—					
10									
11				20					
12				10					
13				6					
14									
15				45.36					
16									
17									
18									
19									
20	ON THERMO -								
21	CYCLER 4°C								
22	51								
23									
24									
25									
26									
27									
28									
29									
30									
31									

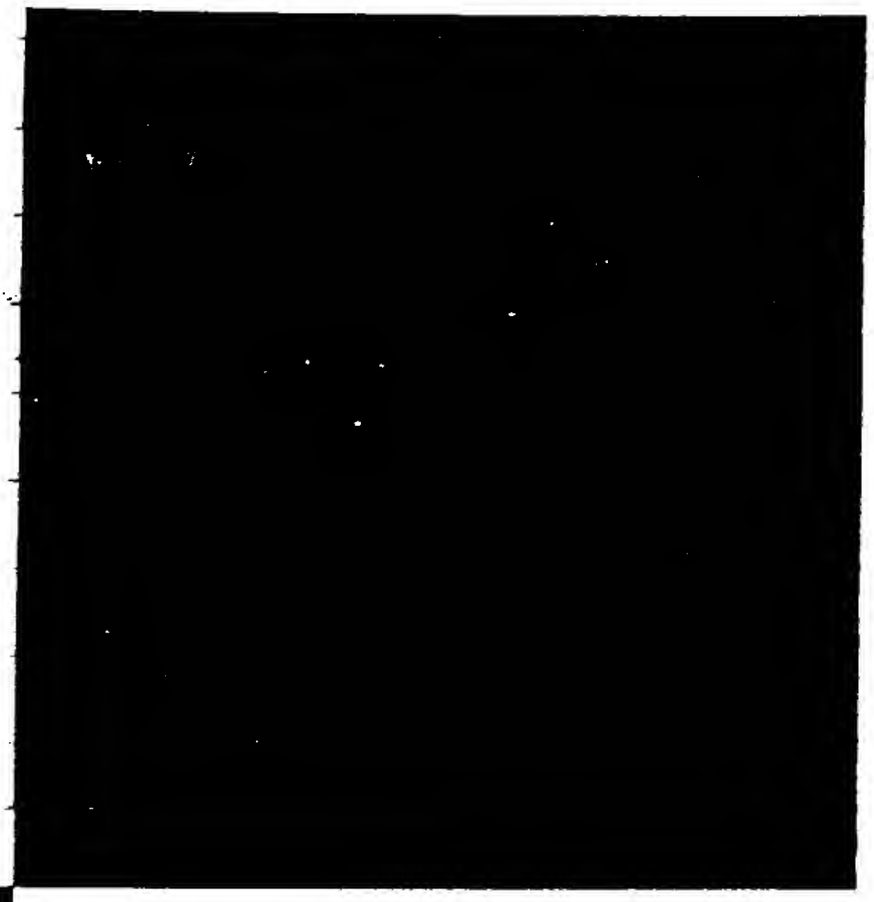


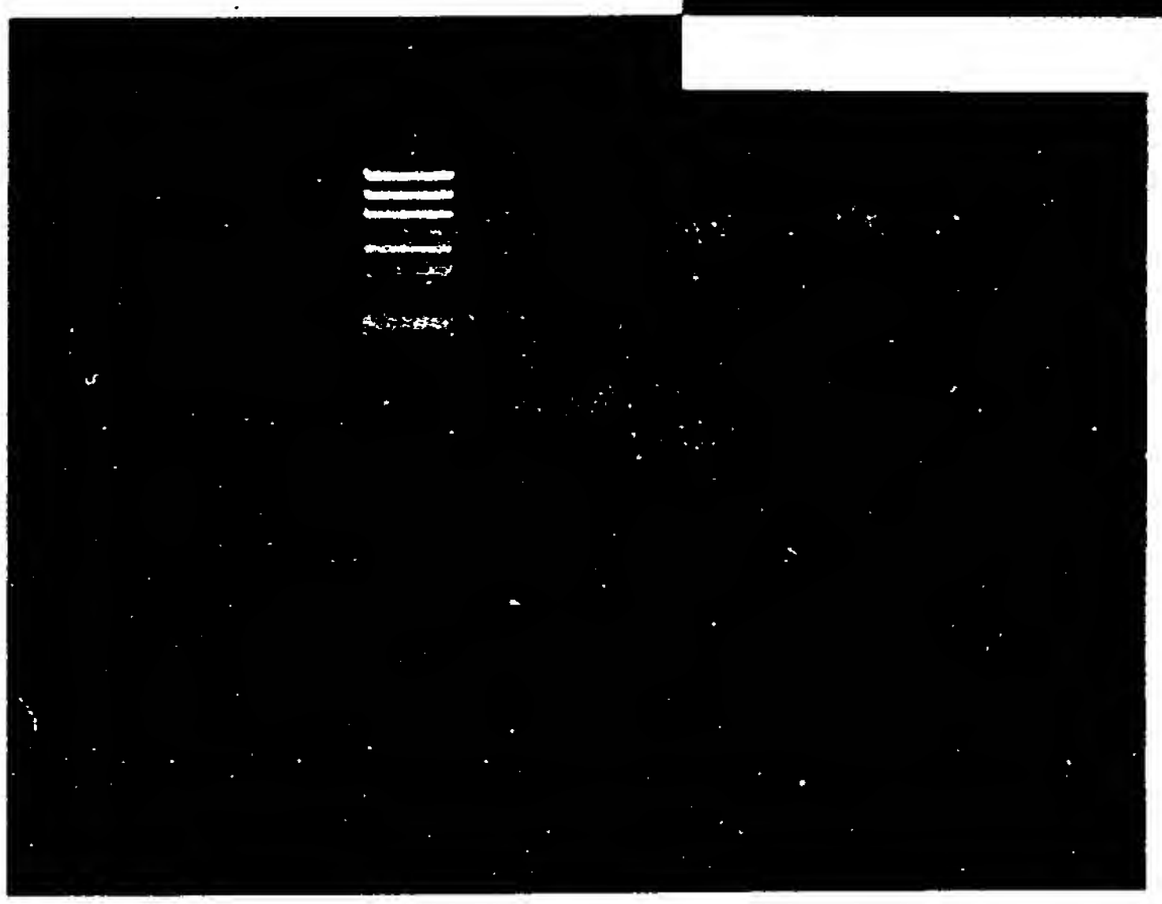


Joel Rainey

PCR 38 (40,421 against cloned DNA) 6/30/8

	1	2	3	4	5	6	7	8	9
1					λDNA				
2	template				1 λ				
3									
4	primers	420	(23.9)		4.2				
5		421	(26.8)		3.7				
6		251	(30)		3.33			420,421	run out
7		252	(51.2)		1.95				
8									
9					20				
10					10				
11					6				
12									
13					49.82				
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									





EFFICIENCY LINE™ 22-206
AMPAC

Joel Raine

Dilutions of Diagnostic DNA

7/9/8

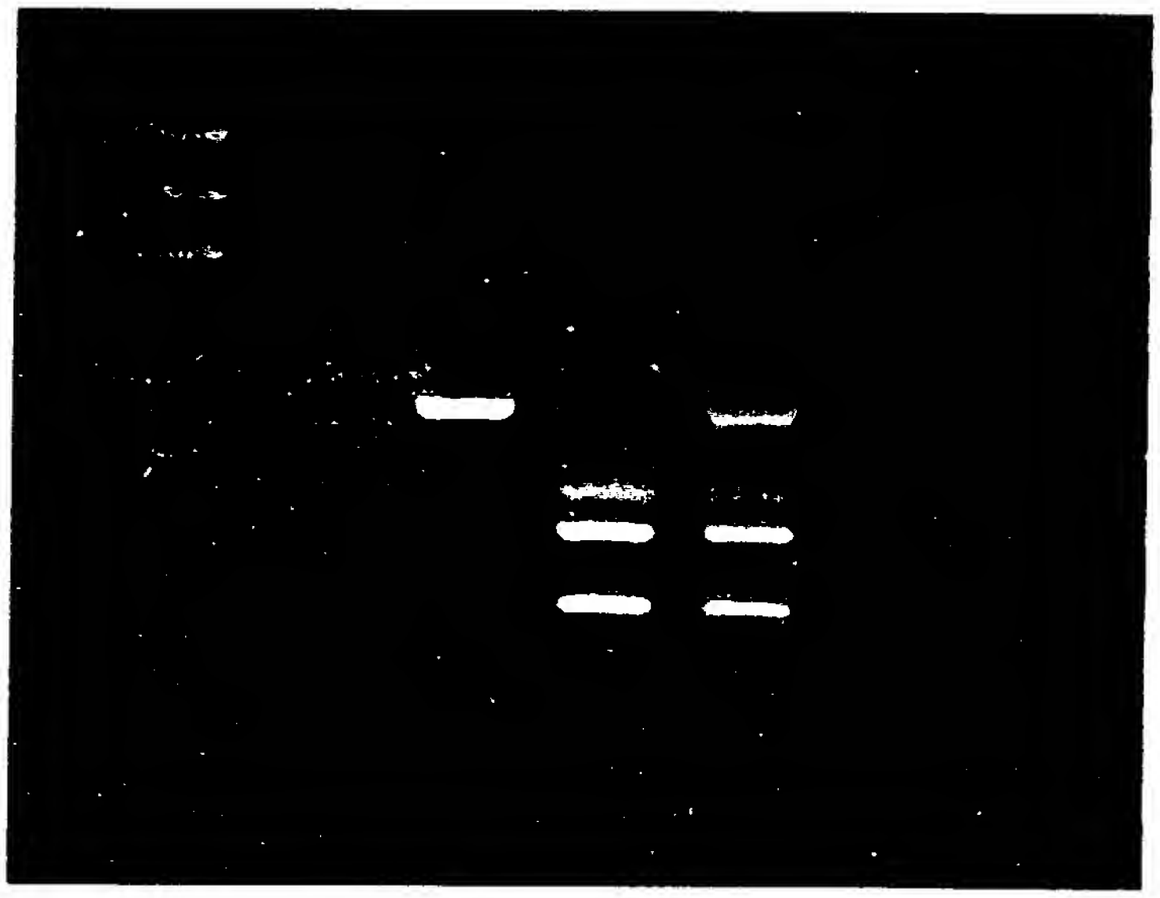
	1	2	3	4	5	6 DNA	7 H_2O	8 A_{260}	9 conc
1	4036								
2	Terry	Frazier		685ng/λ		3.6	16.4	0.031	129ng/λ
3									
4	4066								
5	John	Horner		661ng/λ		3.8	16.2	0.14	
6								0.020	833ng/λ
7	4023								
8	Ryan	Berube		599ng/λ		4.2	15.8	0.090	166.67ng/λ
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

AMPAD · EFFICIENCY LINE · 22-206

Joel Ravier

PCR 39 (Diagnostic) 7/7/8

	1	2	3	4	5 1086	6 4066	7 4023	8 —	9
1					1	2	3		
2	template				3.9	6.0	3.0		
3	H_2O				2.1	—	3.0		
4	primers	221	(136 μM)						
5		222	(150.2 μM)						
6		251	(30 μM)						
7		252	(30.2 μM)						
8		276	(29 μM)						
9		303	(50.3 μM)						
10		395	(123 μM)						
11		396	(91.2 μM)						
12									
13	Mix	1x	4x						
14									
15	221	.74	2.94 ✓						
16	222	.616	2.65						
17	251	3.33	13.32						
18	252	1.95	7.9						
19	276	3.45	13.8						
20	303	1.78	7.1 ✓						
21	395	0.81	3.25 ✓						
22	396	1.1	7.4 ✓						
23		20	80						
24		10	40						
25		6	24						
26	H_2O	44.18	176.72						
27									
28									
29									
30									
31									



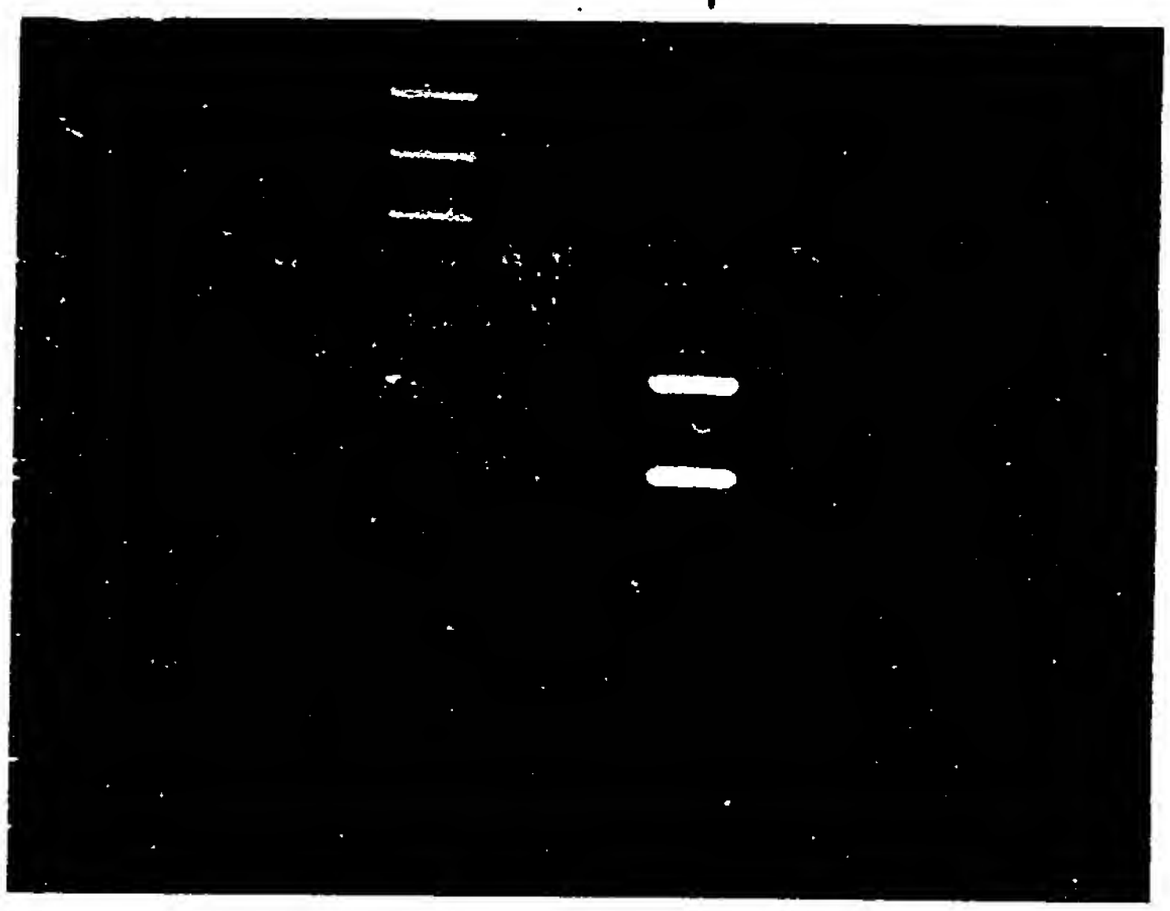
EFFICIENCY LINE 22-206 AMPAD

Joel Ranier

PCR 40! (to test remade 420, 421)

7/12/18

	1	2	3	4 N.Ctl	5	6	7	8	9
1									
2	template			5.46					
3									
4	primers	251	(30)	3.33					
5		252	(51.2)	1.95					
6		465	(19.5)	5.1					
7		466	(17.9)	5.6					
8									
9	5 x Buff			20					
10	DMSO			10					
11	dNTP's			6					
12									
13	H_2O			42.56					
14									
15									
16									
17									
18									
19	41°C	machine							
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									



7/12/8

	1	2	3 A_{260}	4 conc	5	6 H_2O	DNA	8	A_{260}	conc
1										
2	465	28-mer	.303	123 μM		40.0	10.0		.048	19.5 μM
3										
4	466	26mer	.522	228 μM		44.5	5.5		.091	179 μM
5										
6	464	26-mer	.327	143 μM		41.3	8.7		.090	17.5 μM
7										
8	396	25-mer	.270	122.7 μM		39.8	10.2		.052	23.6 μM
9										
10	419	25-mer	.451	205		43.9	6.1		.053	23.6
11										
12	221	27-mer	.186	79.3		34.0	15.0		.059	24.8
13										
14	222	27-mer	0.200	840		35.1	14.9		0.095	23.1
15										
16	395	25		123		39.8	10.2		.059	26.8
17										
18	252	25		208		44.0	6.0		.051	23.2
19										
20	252	25		208		80	20.0		.109	47.2
21										
22	251	26		223		82.1	17.9		.109	47.6
23										
24	276	25		163		75.5	24.5		.092	41.8
25										
26	NCTL.			1.16		90	10		.024	150 ng
27	469								0.098	44.5
28	221	27mer							0.089	37.5 μM
29	396	25-mer							0.089	40.5 μM
30	465	28-mer							0.089	39.4 μM
31	466	26-mer							0.081	35.4 μM

EFFICIENCY LINE 22-206

AMPAD

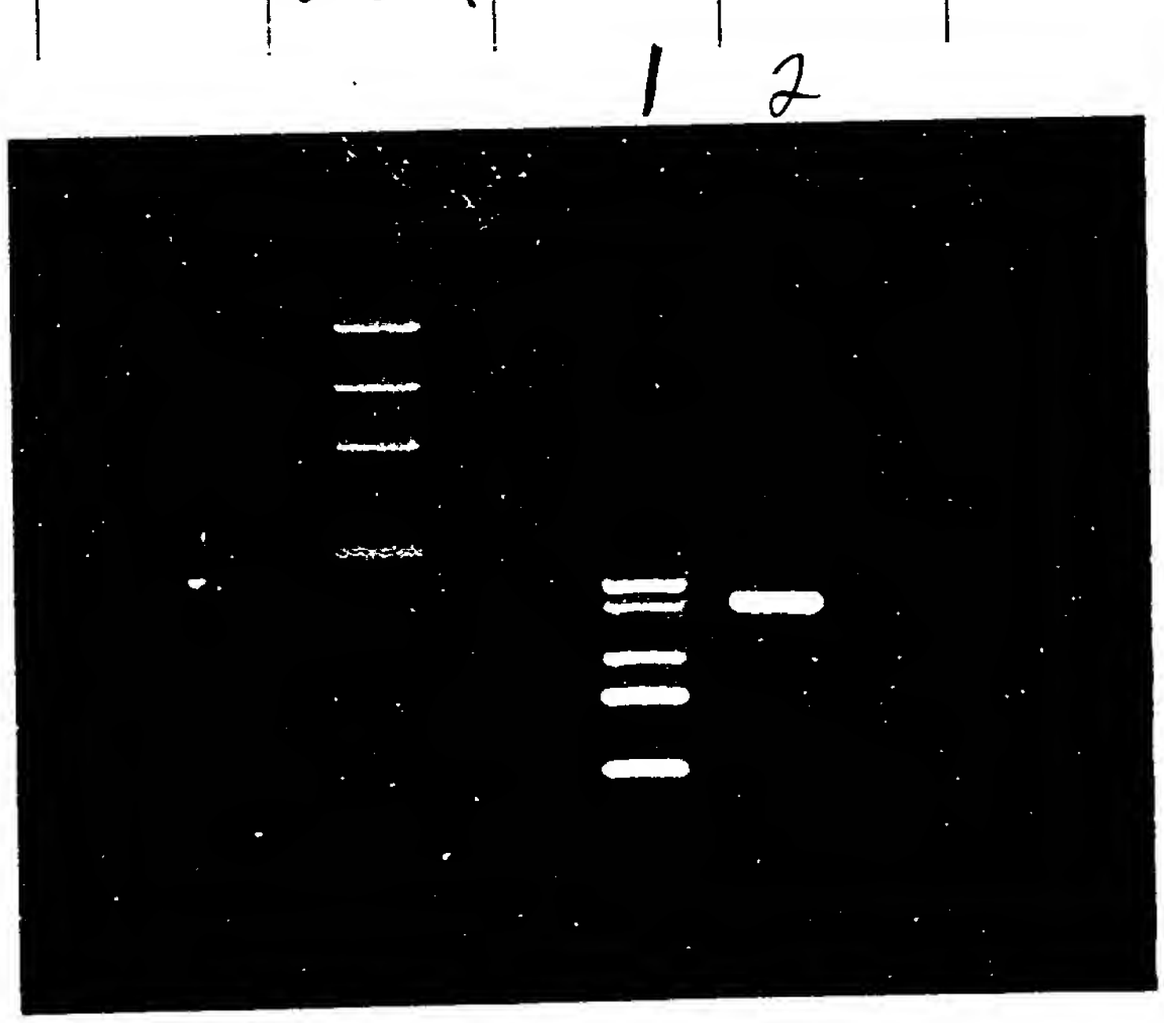
7/13/8

Joel Ravier

PCR 41 (41 primers at 51°C)

9/13/5

	1	2	3	4	N. CTR	6	N CTR	8	9
1									
2	template				5.46				
3									
4	primers	221	136		.74				
5		222	150.7		.66				
6		251	30		3.33				
7		252	51.2		1.95				
8		276	23.6		~~4.35~~ 4.45				
9		303	91.2		1.1				
10		395	~~123~~ 23		~~3.45~~	0.81			
11		396	27.9		3.6				
12		465	19.5		5.1		5.1		
13		466	17.9		5.6		5.6		
14									
15					20		20		
16					10		10		
17					6		6		
18									
19					28.16		47.84		
20									
21									
22	51°C anneal								
23	3:30 ext.								
24	30 rounds								
25									
26									
27									
28									
29									
30									
31									



EFFICIENCY LINE 22-206 AMPAD

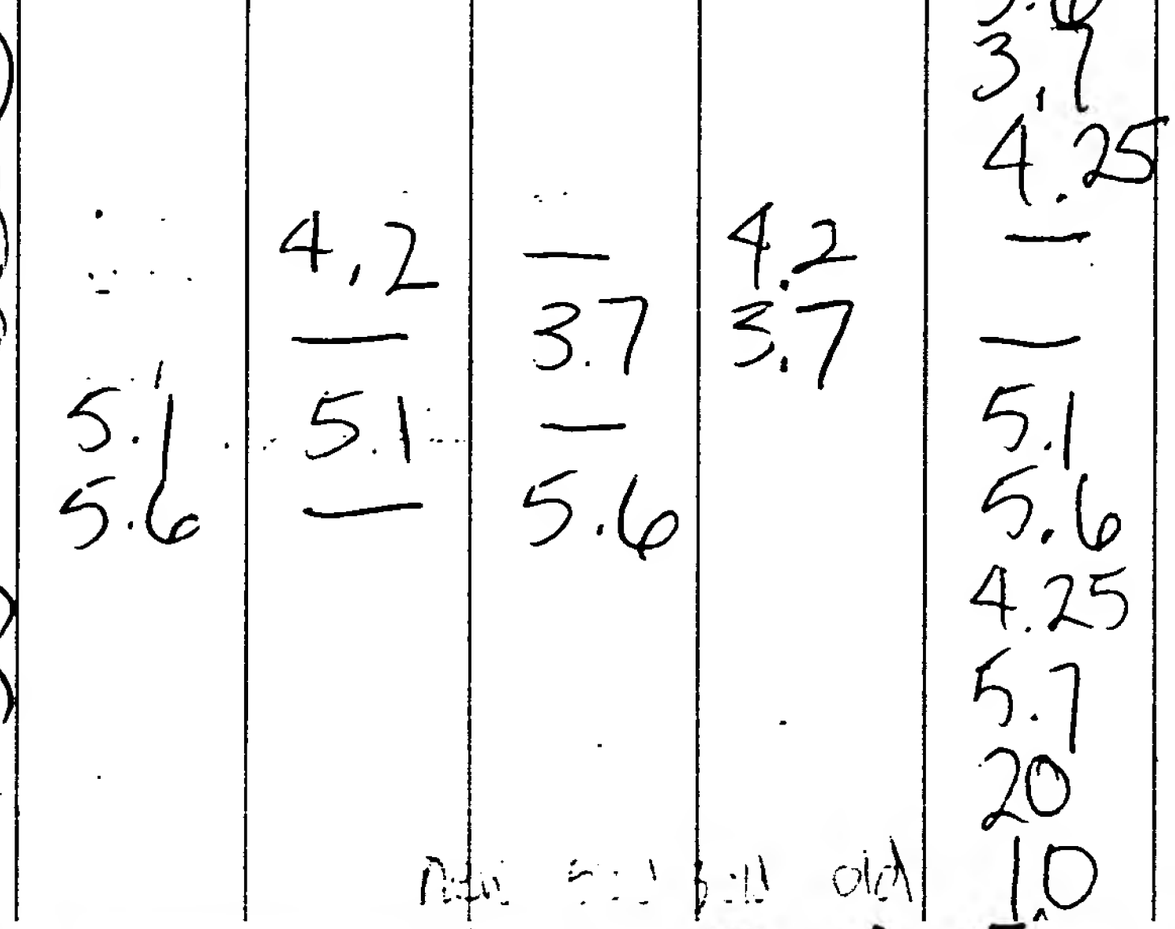
Joel Ranier

9/14/18

PCR 47 (to test 419, 469; Mix/match 420, 421, 465, 466) 5

	1	2	3	4 1	5 2	6 3	7 4	8 5	9 6	X
1				N.CTL	N.CTL	N.CTL	N.CTL	Fetus	—	N.CTL
2	template			5.46	5.46	5.46	5.46			5.46
3										
4	primers	221	(24.8)					4.0		
5		222	(23.1)					4.3		
6		251	(30)					3.33		3.33
7		252	(23.2)					4.3		4.3
8		296	(29)					3.45		
9		303	(27.9)					3.6		
10		395	(26.8)					3.7		
11		396	(23.6)					4.25		
12	5'	420	(23.9)		4.2	—	4.2	—		
13	3'	421	(26.8)		—	3.7	3.7	—		
14	5'	465	(19.5)	5.1	5.1	—		5.1		
15	5'	466	(17.9)	5.6	—	5.6		5.6		
16		419	(23.6)					4.25		4.25
17		469	(17.4)					5.7		5.7
18	5X TaqBuff							20		20
19	DMSO							10		10
20	dNTP's									6
21	H_2O									41.0
22										
23	51° anneal 45s									
24	3:30 ext.									
25	30 rounds									
26										
27										
28										
29										
30										
31										

1 2 3 4 5



AMPAD EFFICIENCY LINE 22-206

Joel Ravier

PCR 43 (All six + diagnostic)

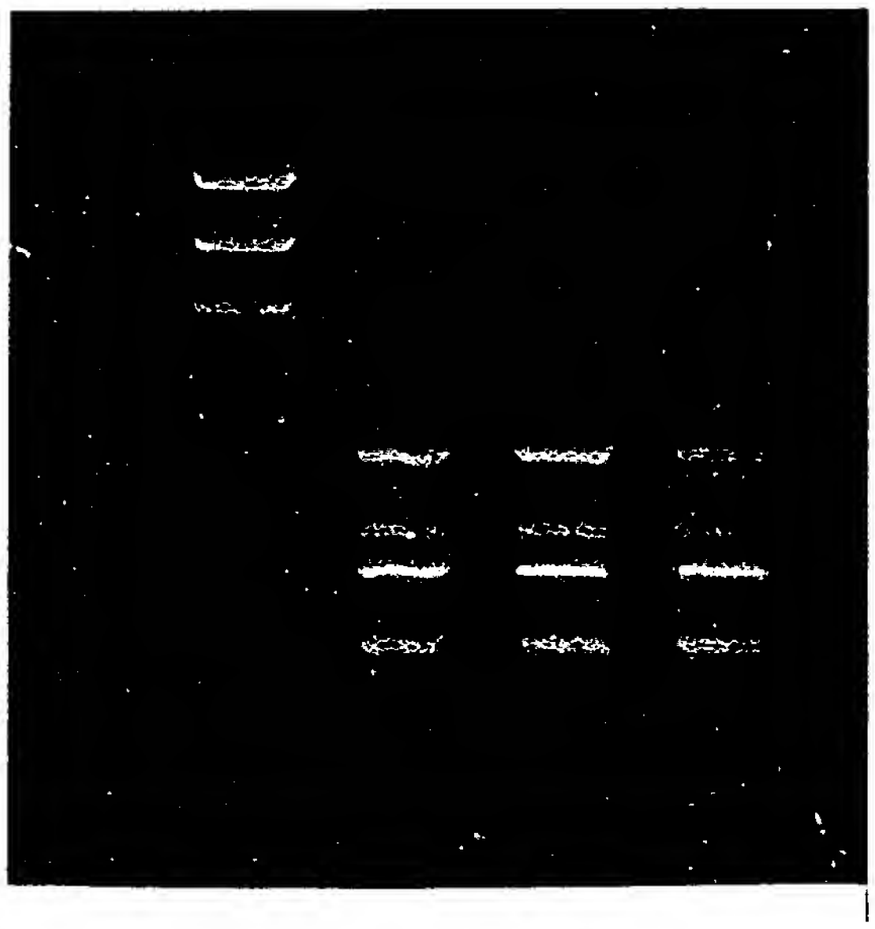
9/15/8

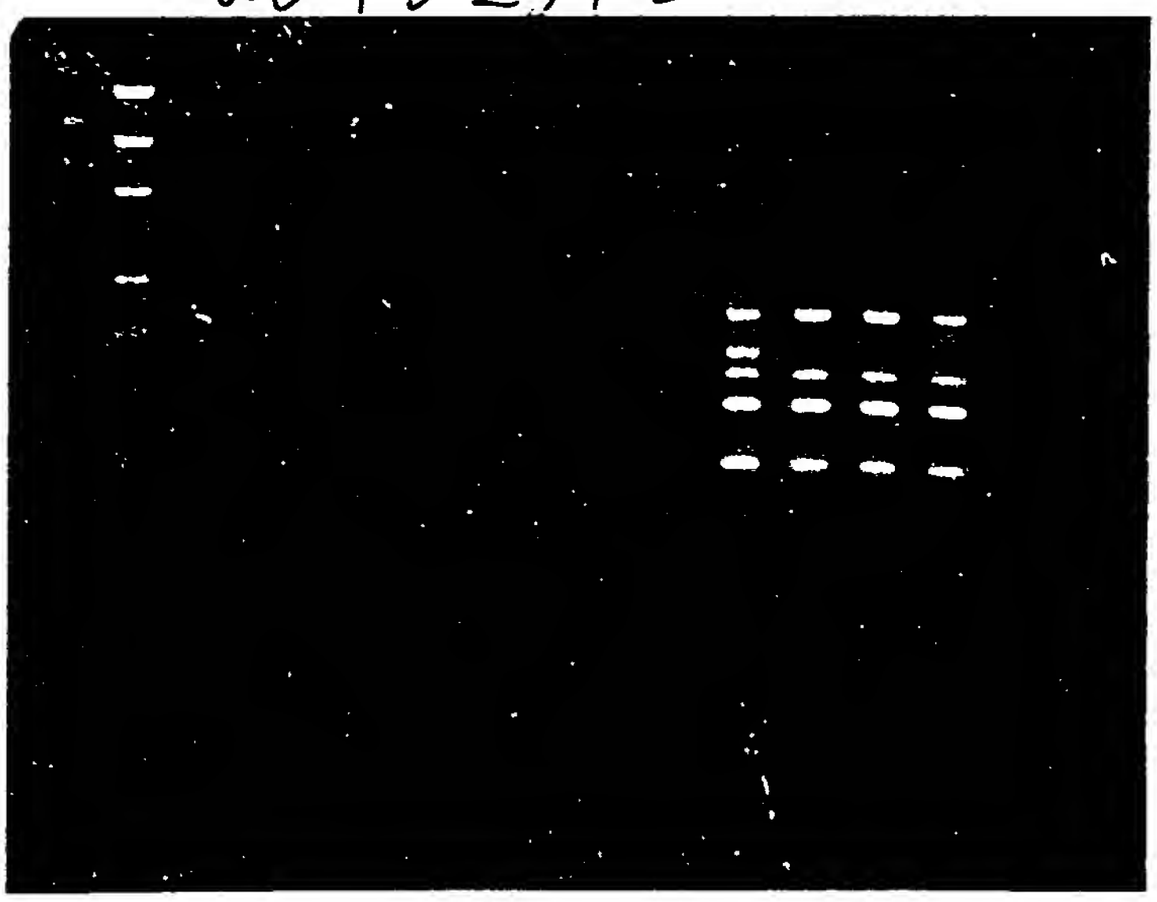
	1	2	3	4 1	5	6 2	7 3	8 4	9 5
1				776		4062	3985	W. Am.	—
2	template			6		4.7	3.08	3.2	—
3	H_2O			—		1.3	2.92	2.8	6
4	primers	221	24.8 μM	4.0					
5		222	23.1	4.3					
6		251	30	3.33					
7		252	23.2	4.3					
8		296	29	3.45					
9		303	27.9	3.6					
10		395	26.8	3.7					
11		396	23.6	4.25					
12		465	19.5	5.1					
13		466	17.9	5.6					
14		469	17.4	5.7					
15		419	23.6	4.25					
16	1/20			6.42					
17	Mix	1x	4x						
18	221	4.0	16						

51°C anneal 45s
ext. 3:45
2x taq

(22) → (26) → 1 2 3 4 5
2 3 4 5 2 3 4 5

(123)





(123)
(228)

7/15/8

	1	2	3	4	5	6	%DNA	H_2O	A_{260}	Conc
1										
2	4062	T.	Eddy	Amnio		259ng/λ	9.8	10.2	.091	
3									.079	492ng/
4	3985	A.	Roybal	Amnio		288ng/λ	8.7	11.3	.099	
5									.062	406ng/
6	4	Danna	Wallage	Amnio		180ng/λ	13.9	6.1	0.169	
7									.092	575ng
8										
9	4062								.026	162.5
10									.017	106.3
11	3985								.047	293.8
12									.026	162.5
13	W. Am.								.042	262.5
14									.025	156.3
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										

EFFICIENCY LINE-22-206 AMPAC

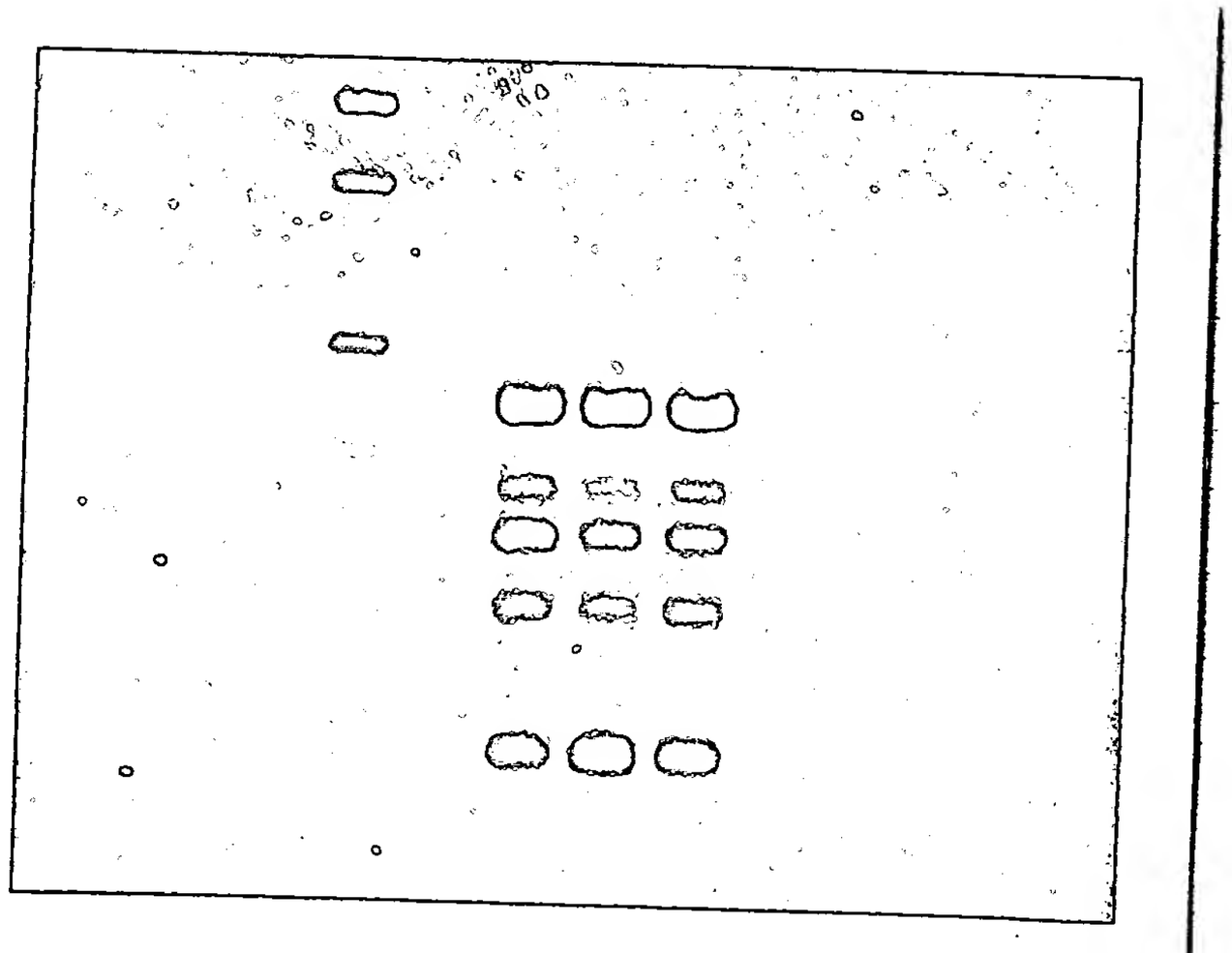
PCR 44 (to adjust conditions for 5th) 9/19/8

	1	2	3	4	5	6 2	7	8	9
1					N.CTL.	N.CTL.			
2	template				3.33	3.33			
3									
4	primers	221	(24.8)		4.0	4.0			
5		222	(25.1)		4.33	4.33			
6		251	(47.6)		2.1	2.1			
7		252	(47.2)		2.12	2.12			
8		276	(41.8)		2.4	2.4			
9		303	(56.3)		1.78	1.78			
10		395	(26.8)		3.7	3.7			
11		396	(23.6)		4.2	4.2			
12		465	(28.0)		3.6	3.6			
13		466	(27.1)		3.7	3.7			
14		469	(17.4)		5.75	5.75			
15		479	(23.6)		4.2	4.2			
16									
17	5x Buff				20	20			
18	DMSO				10	10			
19	dNTP's				6	6			
20									
21	H_2O								
22									
23	Ext. 9.0 min.								
24	(2) 3u Taq								
25									
26									
27									
28									
29									
30									
31									

Joel Ravier

PCR 44 (diagnostic cases) (Evans) (Varma) (Clay) 7/25/8

	1	2	3	4	5	6 1	7 2	8 3	9 4
1						(225)	(112.5)	(231.5)	—
2	template					2.22	4.44	2.16	—
3	H_2O			1x	4x	2.28	—	2.34	4.5
4	primers	221	37.5	2.6	10.67				
5		222	~~23.8~~	~~4.3~~ 1.19	~~17.3~~ 4.76				
6		251	47.6	2.1	8.9				
7		252	47.2	2.1	8.4				
8		276	40.8	2.4	9.57				
9		303	26.8	1.7	7.1				
10		395	26.8	3.73	14.9				
11		396	40.5	2.5	9.9				
12		465	39.4	2.5	10.15				
13		466	35.9	2.8	11.3				
14		464	44.5	2.25	9.0				
15		419	23.6	4.2	16.95				
16	5XBuff			20	80				
17	DMSO			10	40				
18	dNTP's			6	24				
19	H_2O			26.09	104.36	+12.54			
20									
21									
22	3.5 ext.								
23	47°C								
24	Manuel								
25									
26									
27									
28									
29									
30									
31									



7/25/8

	1	2	3	4	5 DNA	6 H_2O	A_{260}	8	9 Calc.
1									
2	(1)	B. Evans (Am)		(524ug/λ)	4.8	15.2	.062		387.5
3							.054	(3-720)	225ug/λ
4	(2)	Asim Varma (affected→)		(406ug/λ)	6.0	14.0	.018		112.5
5									
6	(3)	S. Clay (Am)		(274ug/λ)	9.0	11.0	.037		231.25
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

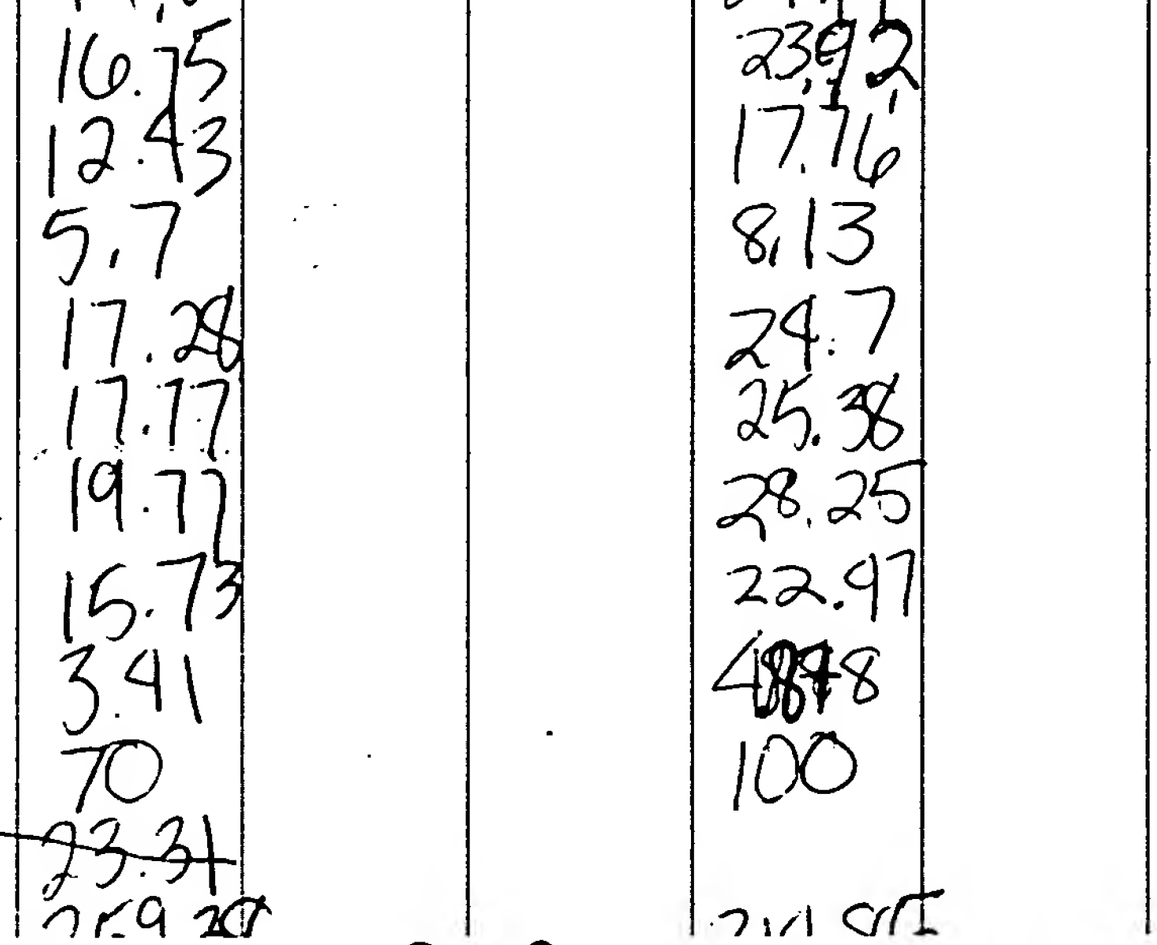
AMPAC · EFFICIENCY LINE · 22-206

Joel Ramos

PCR 44b (storage exper., diag. confirmation + control) 7/21/8

	1	2	3 8	4 9	5 10	6 1+2	7 3+4	8 5+6	9 7	
1			B. Evans	A. Harvey	S. clay	(all 5)	-buff	-dNTP's	all CTL	
2	template		2.22	0.95	2.16	N.CTL.	N.CTL.	N.CTL.	N.CTL.	
3			(225)	(112.5)	(231.25)	3.33	3.33	3.33	3.33	(did not add DNA ex.9
4	H_2O		2.29		2.34	1.17	1.17	1.17	1.17	
5				1x	7x			10x		
6	primers	221	37.5	2.67	18.67			26.67		
7		222	84	1.19	8.3			11.9		
8		251	47.6	2.1	14.7			21.0		
9		252	47.2	2.1	14.8			21.19		
10		276	41.8	2.4	16.75			23.92		
11		303	56.3	1.78	12.43			17.76		
12		395	123	.81	5.7			8.13		
13		396	40.5	2.47	17.28			24.7		
14		465	39.4	2.54	17.77			25.38		
15		466	35.4	2.82	19.77			28.25		
16		464	49.5	2.25	15.73			22.97		
17		419	205	.49	3.41			4888		
18	DMSO			10	70			100		
19	DNA			3.33	~~23.31~~					
20	H_2O				27.9 20			2x1 ctl		
21	Add		~~10.67~~		~~74.0~~					
22	To 1,2,+7		add 6λ dNTP's, 2							
23	To 3+4		add 6λ dNTP's							
24	To 5+6		20 λ buffs							
25	STORED	123	456	-70						
26										
27										
28										
29										
30										
31										

7 8 9 10

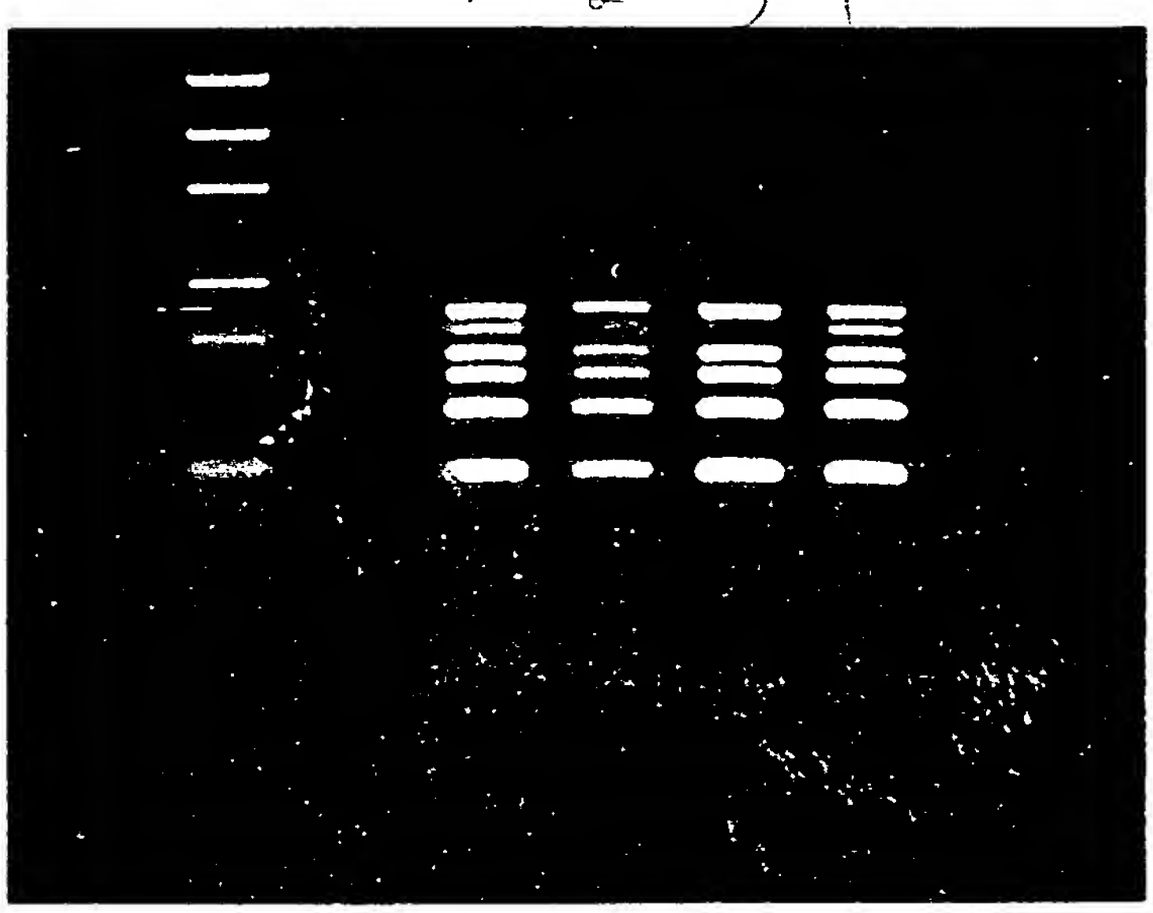


AMPAD EFFICIENCY LINE 22-206

Joel Lanier
JM Clark
7/28/93

PCR 44b

1. (1) positive control
2. (2) B. Evans (Amnio)
3. (3) Asim Varma (Affected ♂)
4. (4) S. Clay (Amnio)



547bp	47.4b	0.5	Hind
509bp	47.1	1.2, 38	"
462bp	30.2	3	Hind
415bp	30.2	1.7	Hind
360bp	9.7	7.5	Hind
268bp	47.4b	4.1	Hind

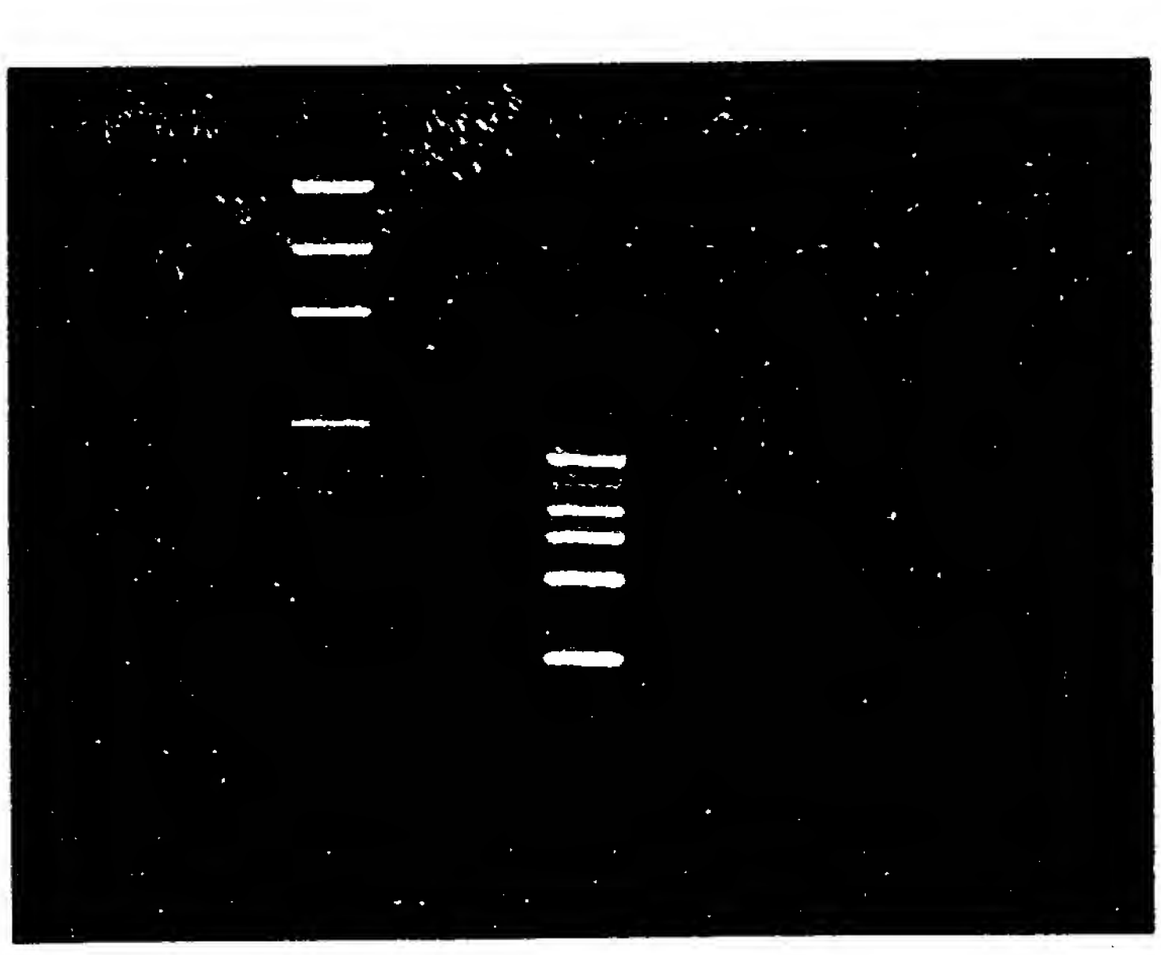
510C

Del Ramier

PCR # 45

7/28/8

	1	2	3	4	5	6	7	8	9
1					N.CTL				
2	template				3.33				
3									
4									
5		221	37.5		2.67				
6		222	39.1		2.55				
7		251	47.6		2.1				
8		252	47.2		2.1				
9		276	41.8		2.4				
10		303	56.3		1.78				
11		395	45.5		2.2				
12		396	40.5		2.47				
13		465	39.9		2.54				
14		466	35.4		2.82				
15		464	44.5		2.25				
16		419	40.6		2.46				
17	DMSO				10				
18	Roller				20				
19	dNTPs				1o				
20									
21									
22	Raised temp. to								
23	55°C anneal								
24									
25									
26									
27									
28									
29									
30									
31									

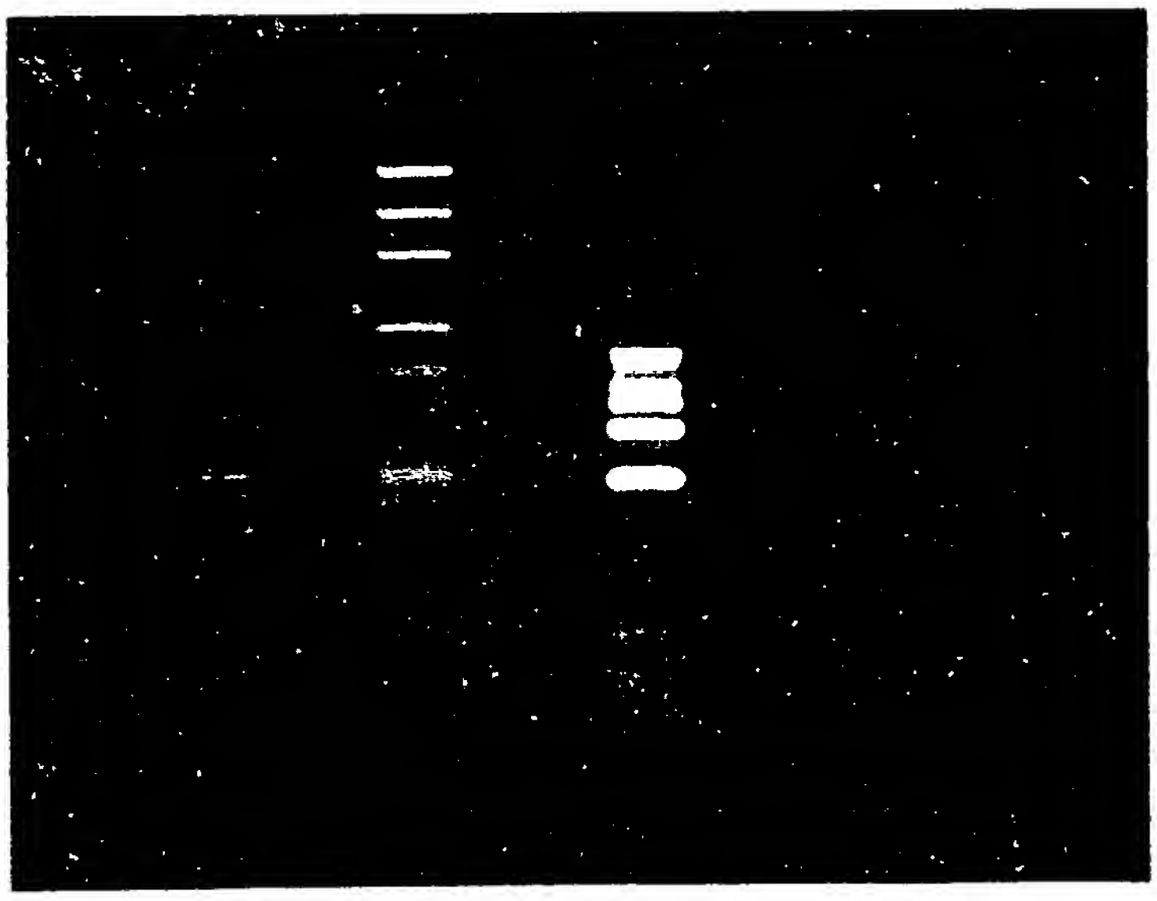


Joel Ravier

PCR 46

7/29/18

	1	2	3	4	5	6	7	8	9
1							N. CTL		
2	template						3.33		
3				1x	3x				
4	primers	221	37.5	2.67	8.00				
5		222	39.1	2.56	7.67				
6		251	47.6	2.1	6.30				
7		252	47.2	2.12	6.36				
8		276	41.8	2.39	7.18				
9		303	56.3	1.78	5.33				
10		395	45.5	2.2	6.60				
11		396	40.5	2.47	7.40				
12		465	39.4	2.54	7.60				
13		466	35.4	2.82	8.47				
14		464	44.5	2.25	6.74				
15		419	40.6	2.46	7.39				
16									
17	Buff			20	60				
18	DMSO			10	30				
19	dNTP's			6	18				
20									
21	H_2O								
22	Add	96.67							
23									
24					35				
25					rounds				
26									
27									
28									
29									
30									
31									



Joel Kanter

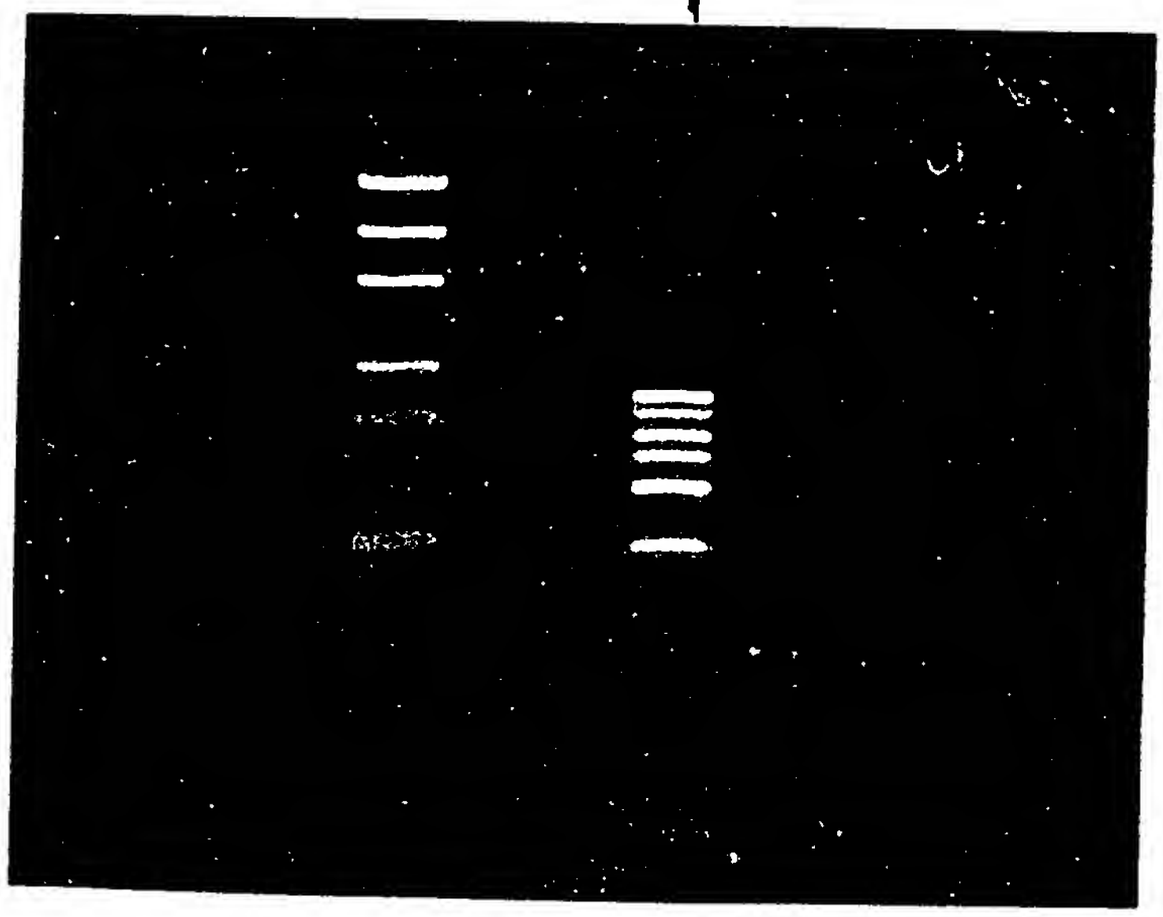
PCR 47 (to raise conc. of 44.1 amplicon) 7/31/8

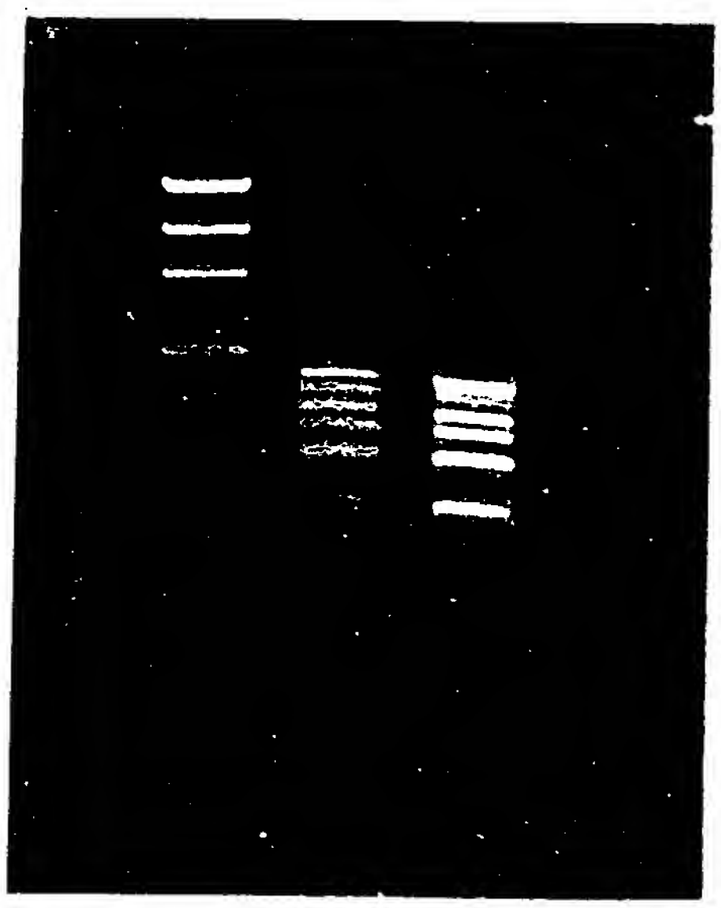
	1	2	3	4	5 1	6 2	7	8	9
1					N.CTL	N.CTL			
2	template				3.33	333			
3									
4	primers	221			2.67	"			
5		227			2.56	"			
6		251			2.1	"			
7		252			2.12	"			
8		276			2.39	"			
9		302			1.78	"			
10		395			2.2	"			
11		396	(44.5)		2~~.5~~	"			
12		465			2.54	~~3~~.81			
13		466			2.82	4.23			
14		464			2.25	"			
15		419			2.46	"			
16									
17	buffer				20	"			
18	DMSO				10	"			
19	dNTPs				6	"			

50% more 465,466

(20) (19)

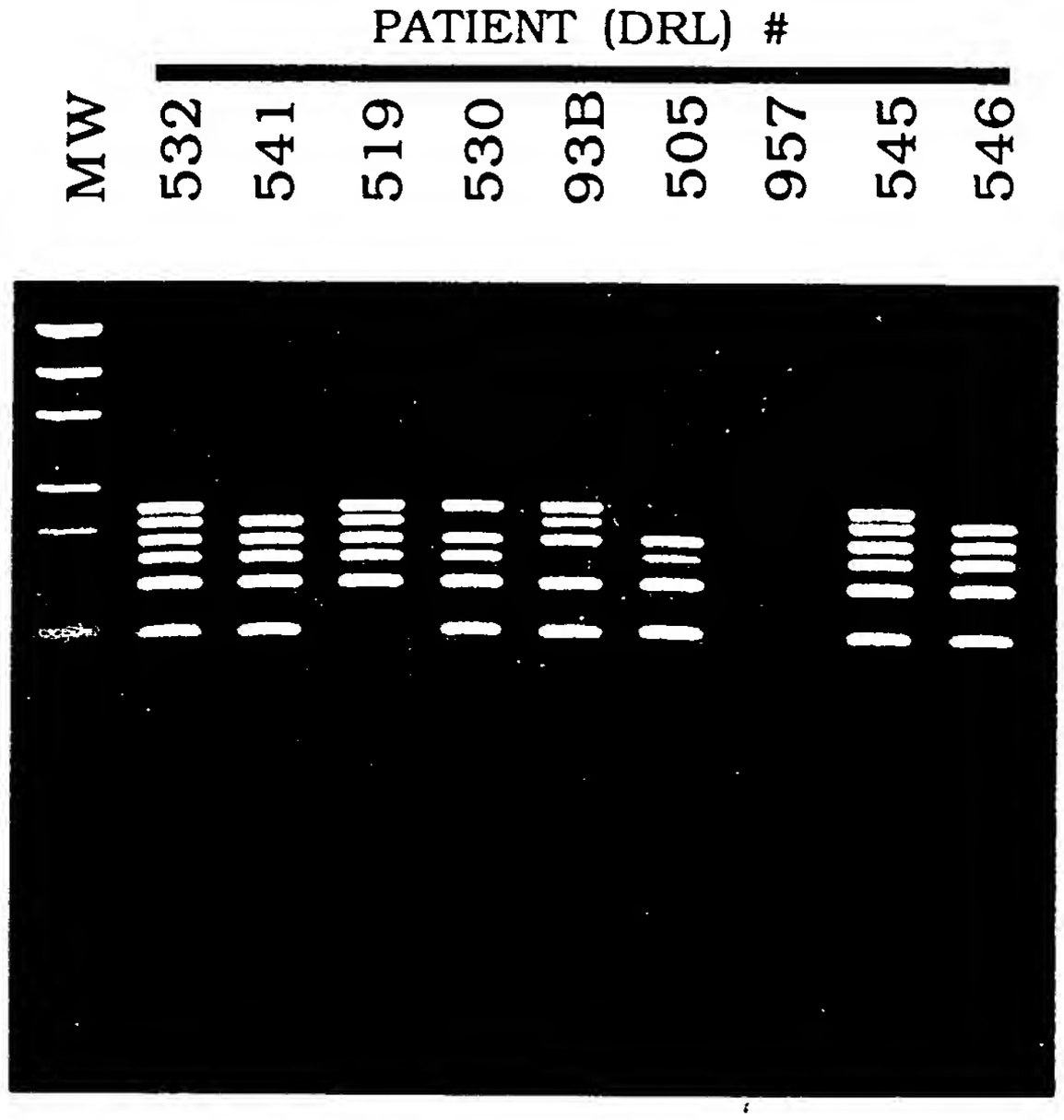
2^{25} 2^{30}

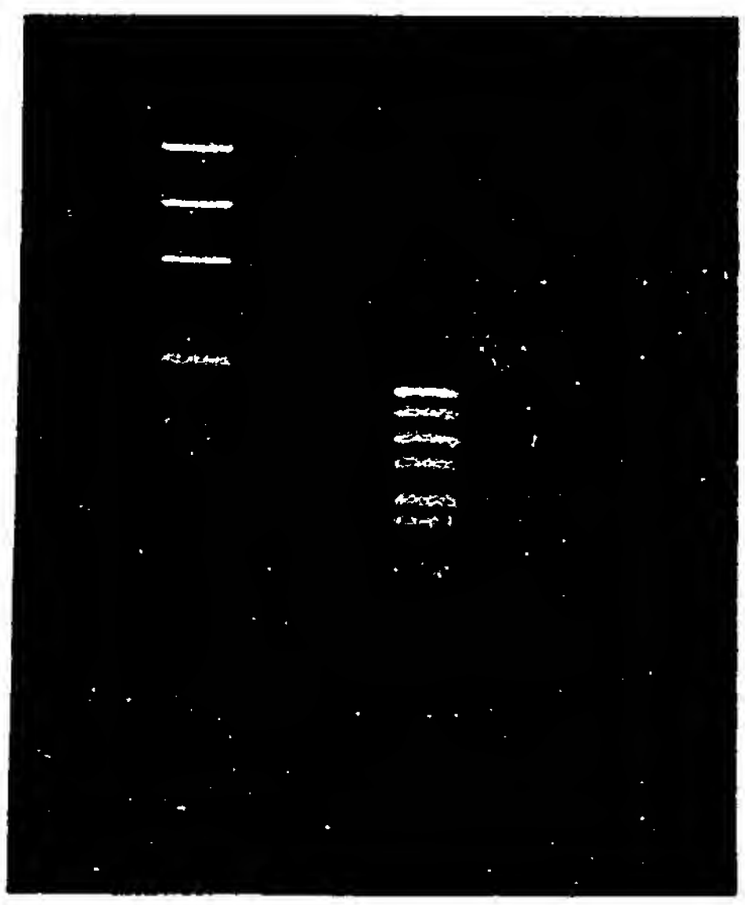




25 rounds 20 1 55°C

Joel Kanter





Re-screen of 44-1 gen. clones 5/12/8

Hong's screen of 4x4 Library λDASH

- need to re-purify 5 picks:
  1C, 2A, 7B, 7A, 9B
- plate 10 + 100λ of 2° picks
  (2nd pick of 20 - row 3 Hong's box)
  got 1-100 on 10λ plates
  lift all except for 1 too lo + 1 too hi
- pre-hyb O/N 50% F

Label

44-1 ~20ng ½ rxn 11:15 →
0.6 HcII frag 63-1 ~20ng " "

Hyb in 40% F

TCA↓ cpm:
1λ/100λ: 44-1 → 20x10⁶ cpm
0.6 → 28x10⁶ cpm

lifts - 7 filters
12mls - use 60λ 44-1

also hyb Joel's blots of his 5 cDNA clones
1) cut c̄ SstI-Kpn 2) cut c̄ EcoRI
⊕ with 63-1 2.5kb HC - check for hyb to 0.6kb HcII 63-1
10mls 40% F c̄ 50λ probe

PCR CASES DMD 5/18/[illegible]

<u>family</u>

- Russel Jordon

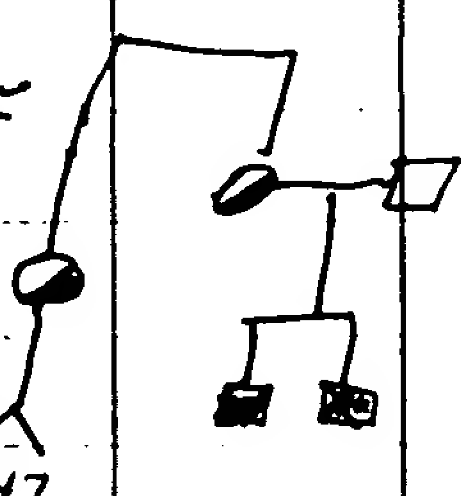
483 Jordon - Aff. male XJ1.1 + 9.7 deletion
male fetus not affected
- do Aff. male + fetus only

43B Ruggiero Aff male (Nicholas Posay) 449, 638
87.1-15 deletion
male fetus unaffected - 3653, 3666
- do fetus + aff. male

ODD severe becker deletions - Randy + James Branche 3565 + 3566
469 Roussean 2 Bros. c̄ severe BMD/mild DMD
deletion of 9.7 Exons 3-7
but funny pattern at 7.5 kb band
similar to 2nd family
PCR for 'Innadel' exon only + 0.5 HB [illegible]

→ aff. male c̄ same deletion DNA #1504

Roussean



3647

also 3rd case c̄ similar 9.7 deletion + same funny pattern AT 7.5-8.5 kb: Watkins (3685), Shannon ♀; Alfred (aff ♂) (3758) + fetus (3722)

<u>Previous PCRS</u>

1st PND - fig in UCLA manuscr: 461, green [Craig Spinnato aff. male]
♂ fetus not deleted

2nd twins ♀♀ 396 Mecio - normal pattern

1µg

MEMO

Jeff X4777 needs 0.5µg DNA on below. - (Please call him when ready)

43B Nicholas Posey 449, 638

- Male fetus Ruggiero — recd 1988. 3653, 3666

469 Brunche, Randy OR James. 1µg/µl 3565, 3566

- Lynn Rousseau - 3560, 3561
- Male fetus Rousseau 3647
- Mary Richardson 3569, 3582

485 - Watkins, Sharon 3685

- Watkins, Alfred - 3758
- Watkins, male fetus 3732?, 3796? Same

grendel CR

Deletion Summary multiplex figure 5/19/88

	1	2	3	4	5	6	7	8	9
1									
2	DRL#	DNA#	NAME		deletion				
3	42	665*			9-7-730-2	7.5, 10.5, 4.2 only	⊕XJ1.1		
4	93	660*	Hadicon		⊕XJ1.1; 7.5; Δ30-2:	10.5→87.15;	⊕87-27, 25, 30		
5	~~184~~ 156	1011	Eric Cronick		Δ47 46-44-1?	0.5-1.6	BMD		
6	24	641*	Scott Jones		+XJ1.1; Δ9-7-30-2:	7.5→87.15;	⊕87-27, 25, 30		
7	?	957*	John Brandt		Δ754-J-Bir; Δ	0.58 47.46	3' breakunknown		
8		776			pos. control for region of interest;	?→			
9									
10		* = Tested with XD-1							
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

5/20/88

1	2	3	4	5	6	7	8	9

776 pos. control

DRL # 42 665 — mandel + (-7.5, +10.5, 4.2)

DRL # 93 660 — 87.15 Haddicon -10 +7.5

1011 — 0.5 Eric Crimink, DRL #156 BAD

DRL # 24 641 Scott Janes - mandel, - 87.15 (-10, 7.5)

✓ JB all 3 DNA #957 BRAZAEL Δ 754, Δ J-BiR ▲

(156) ✓ 1011 deleted 474B 0.5 → 1.6

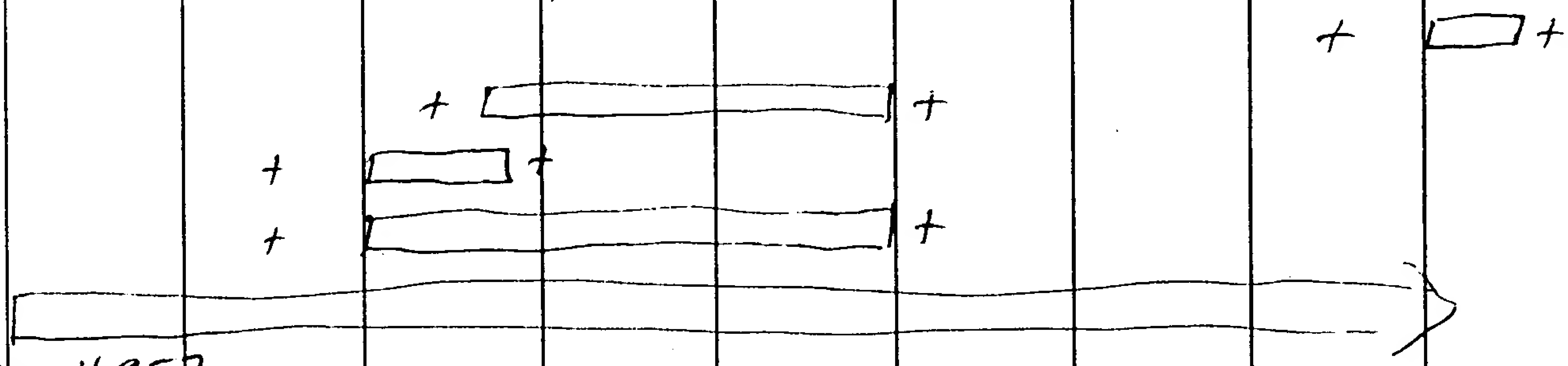
✓ (93) 7.5 (+) | 10.5 (−) → ? -87.15; + 87.25 (27) + 30

✓ (42) Δ 7.5, 10.5, 42 only

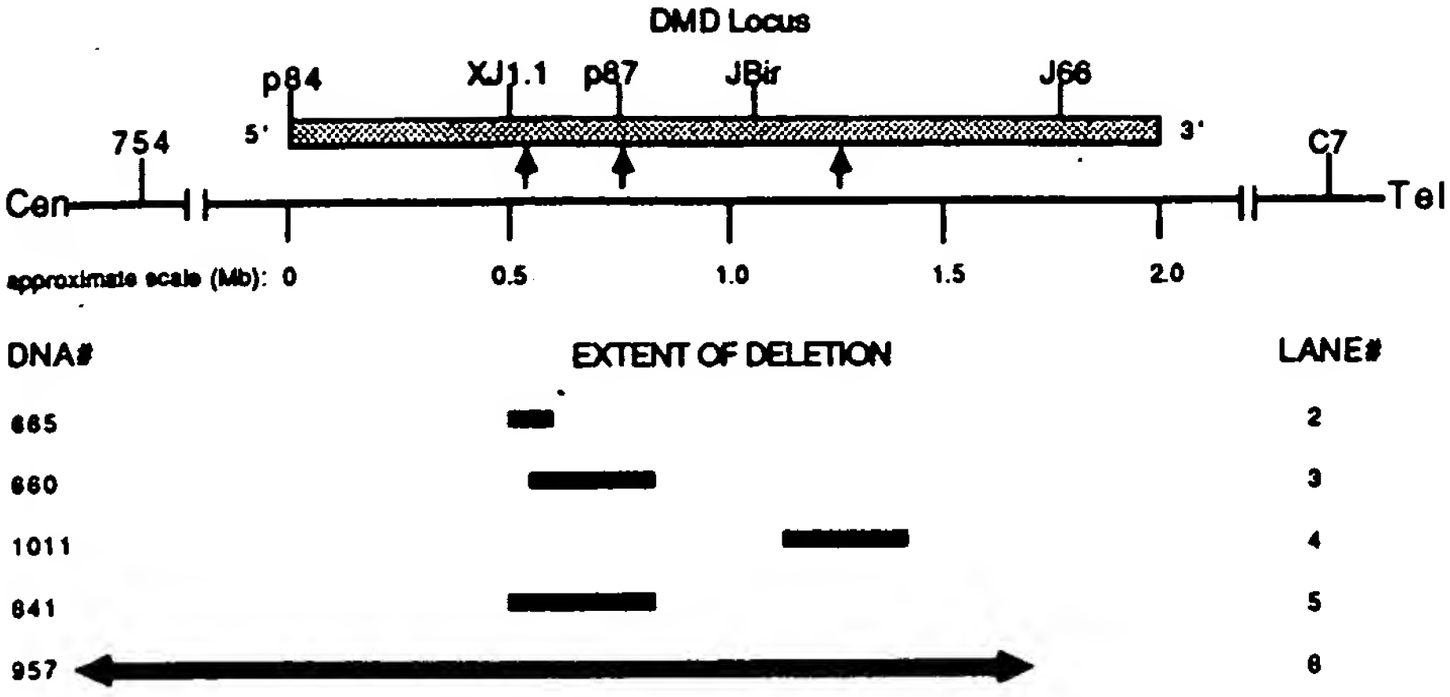
(24) (−) 4.6 | (−) 7.5 → (−) 87.5; (+) 87 27, 25, 30 →

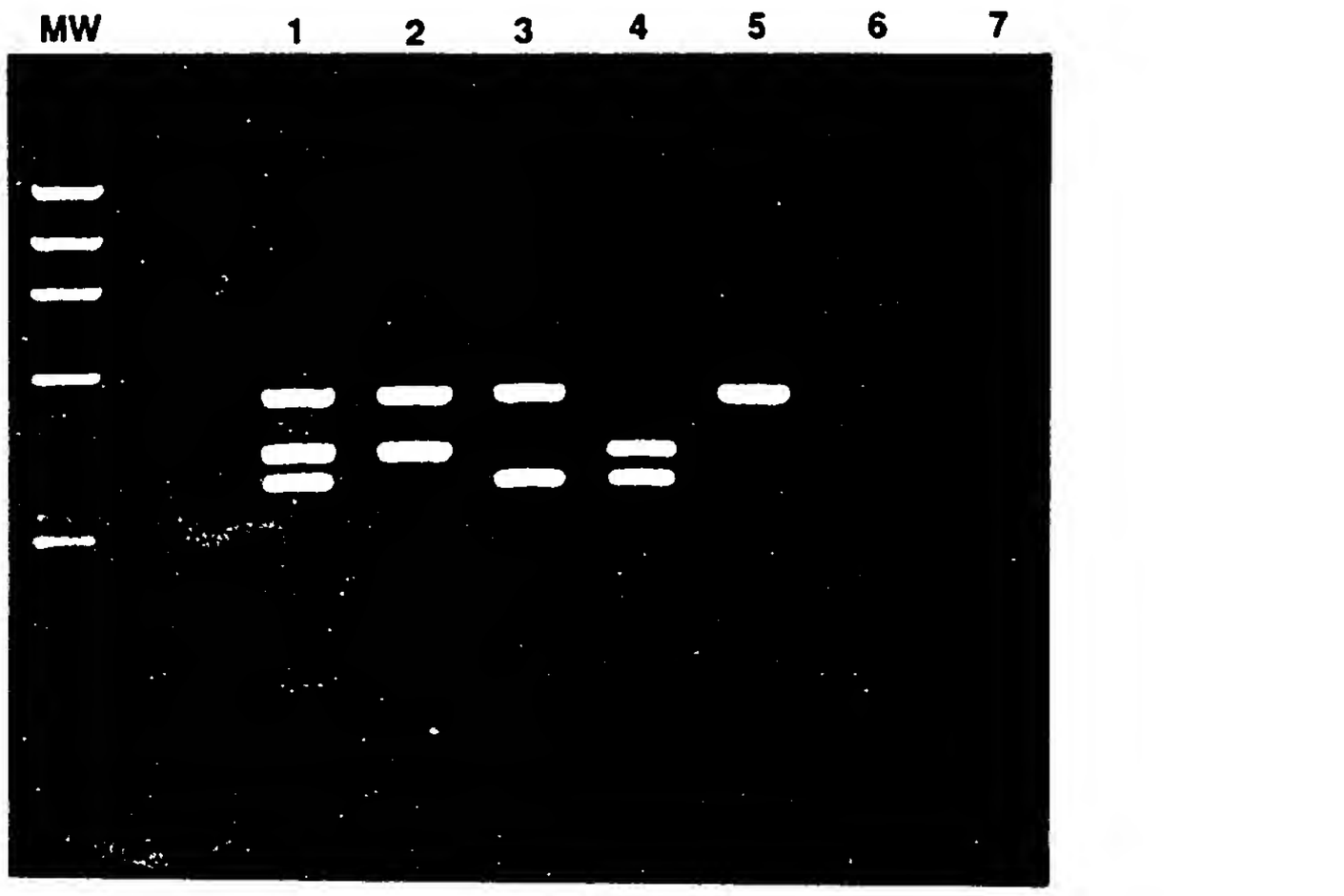
754 XJ1.1 man PERT 1 8 15 27 30 J-BiR

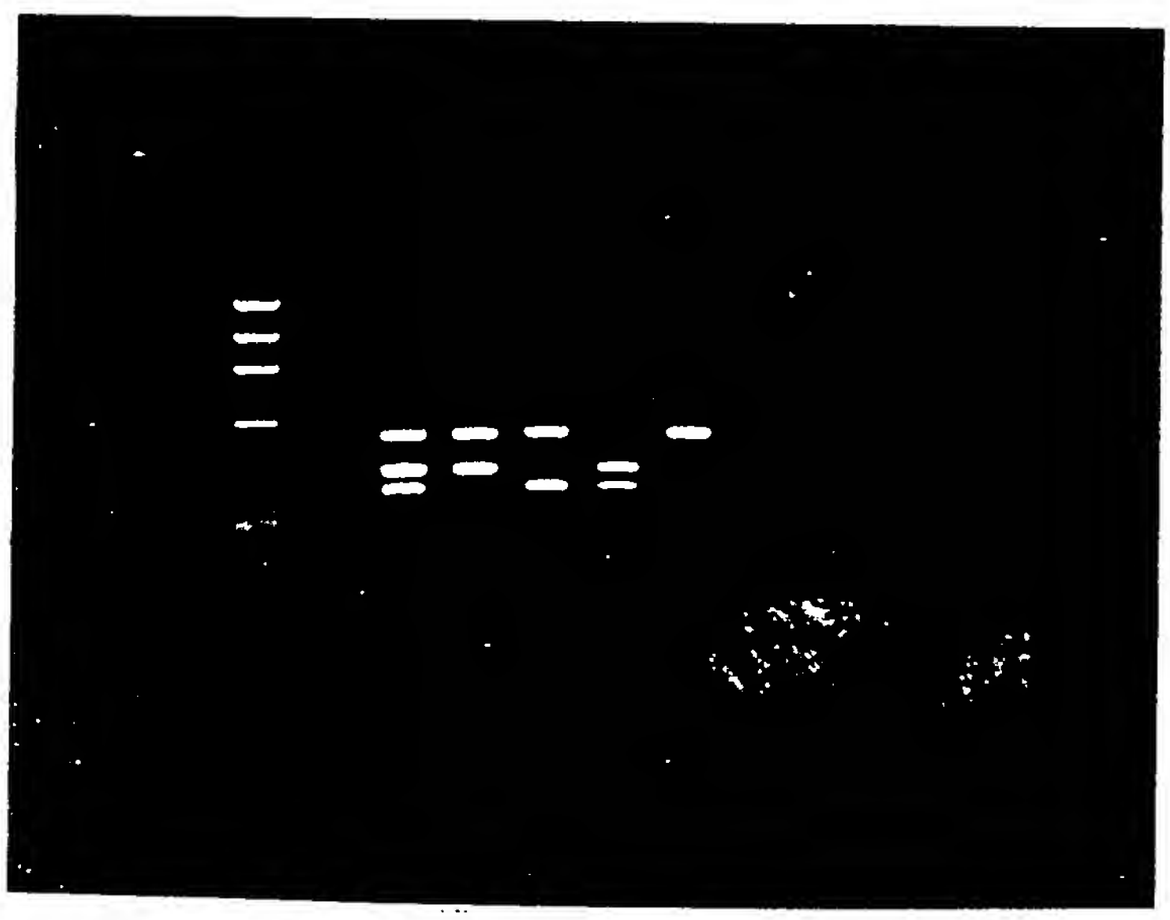
1011
93
42
24
JB → DNA #957



5/20/88

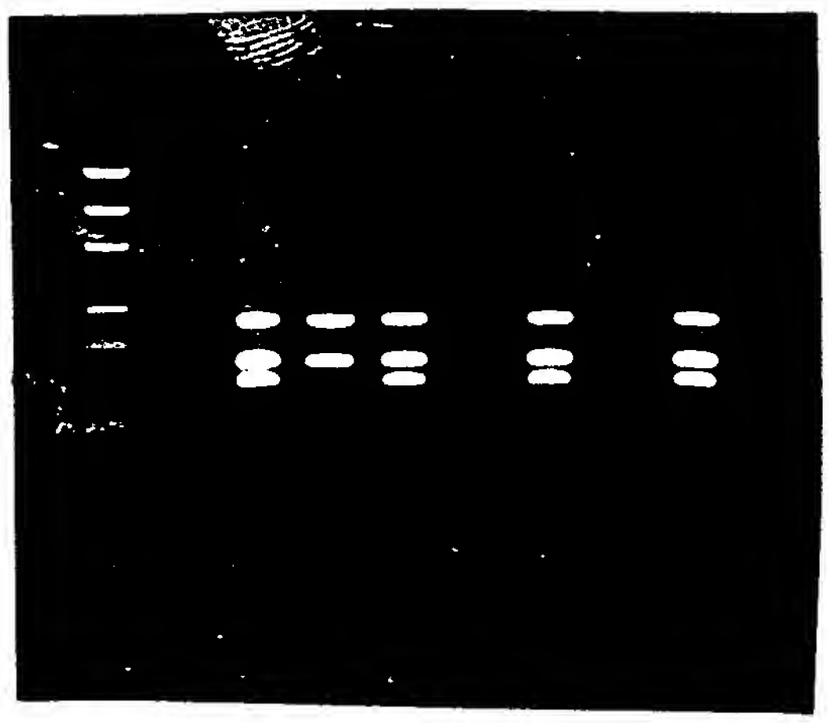

DMD Locus
p84
XJ1.1
p87
JBir
J66
5'
3'
754
C7
Cen
Tel
approximate scale (Mb): 0
0.5
1.0
1.5
2.0
DNA#
EXTENT OF DELETION
LANE#
665
2
660
3
1011
4
841
5
957
6


MW
1
2
3
4
5
6
7



5/24/88

	DRL# 483				DRL# 43B		
MW	CF	AM	MF	X	CF	AM	MF



6/3/85

Joelle –

→ PCR on all 4ff. male DNA —

473 Surrette
484 Miller
505 Young
513 ~~[illegible]~~ Capuo
514 Reisbeck?
519 Van Zandt
520 Stone
521 Worth.
522 Cox
523 Creighton
524 Buchanan
22B Derry
531 Roybal

See me
D. Gaskey

PAT WATCH

6/2/88 DNA samples for PCR on DMD males

	DNA #	NAME	DRL #
* 1st Priority Group	346	George Derry, Jr.	22B
	472	Thomas Davis	70B
	3934	Shane Worth	521
	3955	Alan Cox	522
	3920	Douglas Hazelton	523
	3944	Billy Buchanan	524
	3948	Andrew Royse	531
	3929	Keith young III	505
	[redacted]	[redacted]	[redacted]
2nd priority Group	3940	Scott Miller	484
	3860	Donald Capello	513
	3880	Wayne Noon	514
	3895	David Van Zandt	519
	3950	Matthew Stone	520

6/7/88

Dot-blot hyb. of 44.1 3.8 kDND cDNA clones

1. 5λ mini-prep DNA , pUC8 control.
   - add 4λ 5M NaCl
   - add 1λ 1M NaOH
   - boil 2', ice
   - add 1λ 100% etk.

2. Pre-wet nylon membrane ē H_2O
   - spot DNA on filter, 5λ/attempt
   - air wash gently in 2×SSC
   - air dry

3. Pre-hyb 1hr, 50% F, add extra SDS to 1%

4. Hyb ē 44-1 ~3 hrs.
5. wash, hi-string.
6. expose to film.

6/8/88

44-1 3.8kb HJ cDNA

US. 44-1 cDNA

15kb exp.

Labelling of 44-1 oligomers   7/6/88

	40 mers not purified	1/5000 dil. 260	280	260/280	1/5000 conc. mg/ml	total yield
442	10kb exon	.496	.276	1.80	.016 mg/ml	4mg.
443	3.1kb exon	.444	.222	2.00	.015 mg/ml	3.66 mg
444	3.7kb exon	.496	.275	1.80	.016 mg/ml	4 mg

dissolve in 50λ TE (1/2 syn. off column) dilute 1/500, + 1/5000

1/5000 dilution each is 15-16 ng/µl (ng/λ)

26ng = 1pM end.
5pM end = 130ng = 8-9λ

Label each

DNA	3λ
10X kinase	1λ
T4 kinase	1λ
γ-ATP P^{32}	2λ
H_2O	4λ
	10λ

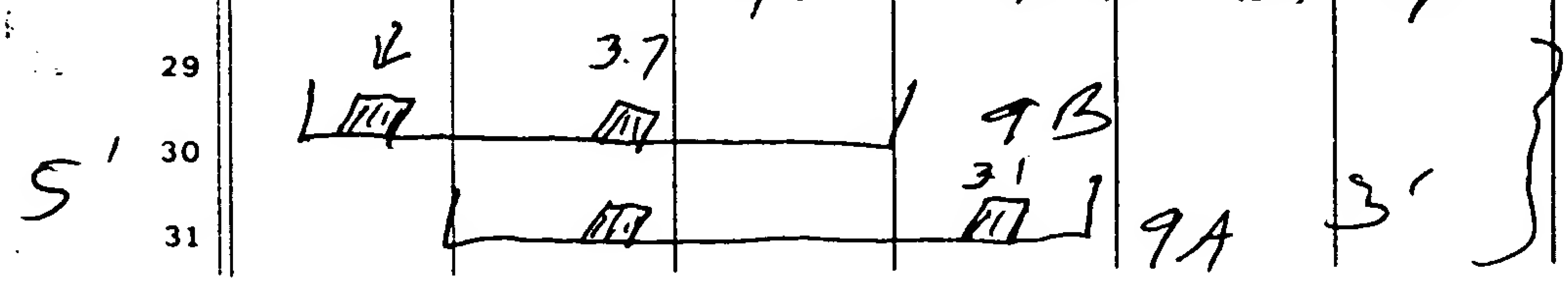
after 1hr 37° add 90λ formamide dye + load 4λ on a 12% acryl. gel - save rest for hybs.

Hyb to old blots of Hong clones 9A + 9B

443 - Lit up 9A Eco band that lit cDNA nothing on 9B (3.1kb 9B)

444 - Lit up new Eco band on both 9A + 9B wasn't lit cDNA, different than 9B cDNA band (3.7kb)

442 - none lit up (10kb)



possible exon structure

exon's cloned 7/12/88

5' → 3'

B.P. Amp'd

① 360 1) 9.7 => 7.5 kb HindIII exon 8

② 415 2) 87.15 Adjacent exon [30.2, 7.6 kb H3] exon 17

462 3) not Tested yet, 87.25 Adj exon [30.2, 3 kb H3] exon 19

④ 268 4) 47-4b 4.1 kb H3

③ 547 5) 47-4b 0.5 kb H3

506 6) (partially tested) 44-1 [1.2 + 3.8 kb H3]

---

cloned, sequenced, no oligo's yet

7) exon 12 (30.2, 4.2 kb H3)

3)

cloned, being sequenced

A) 44-1, 1.6 kb H3
B) 44-1, 3.1 kb H3
C) 44-1 3.7 kb H3 - prob. not confirmed

---

freq. of detection:

1,2,5,1,2,3 = 35% k-del, 45% Lesch

1,2,4,5 => slightly higher

1,2,4,5,6 => 75%

1-6 => 80%

1/10,000 dilution | OM 471, 472, 473 | Seq primers 44-1 region

	260	280	260/280
471	.057	-.010	2.03
472	.114	.054	1.30
473	.099	.050	1.12
BL	0.000	-.038	

	0	-.042	260/280	conc.	yield
471	~~.041~~ .141	.041	1.58	4.65 ng/μl	4.65 ng
472	.171	.072	1.43	5.69 ng/μl	5.6 ng
473	.120	.048	1.33	3.96 ng/μl	3.96 ng

dissolved in H_2O; 1/10,000 dilutions prepared for sequencing

9A + 9B prep 1/4/8

- plate 4 plates stirred 7/13/8 5 days

9A ~ 4×10^7/ml

9B ~ 8×10^7/ml

replate ~ 100k/plate

2, 9B, 4, 9A

AECAACCTCTAGCAATATCCATTAEC

C16

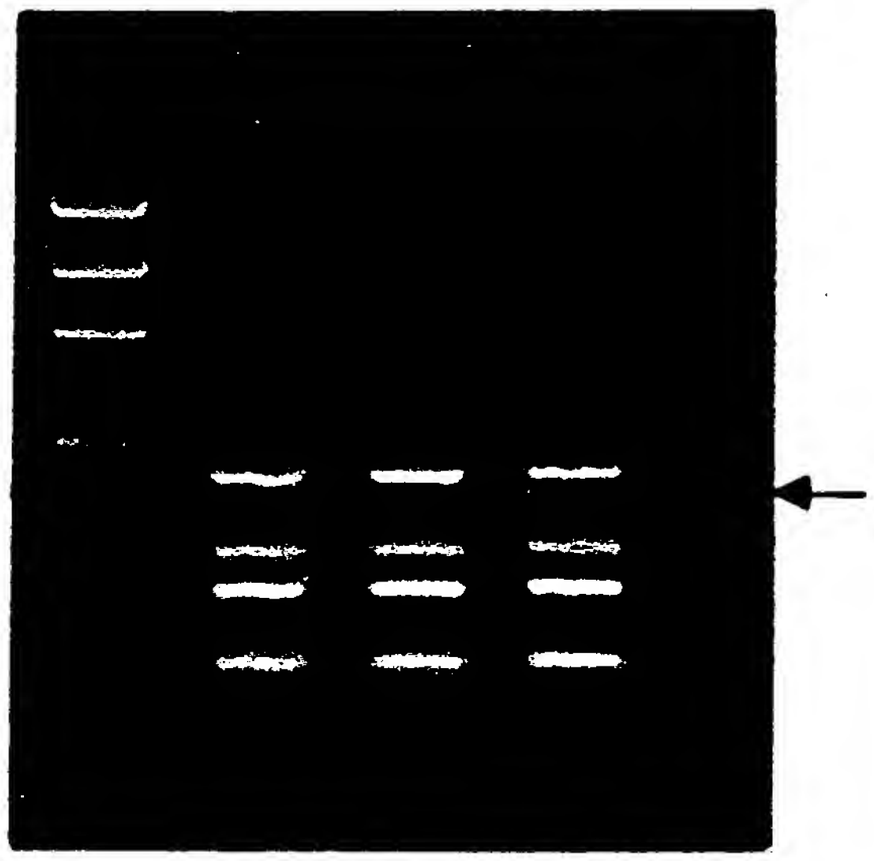
GTC

TC7766ACCTXATA

PRENATAL DIAGNOSIS of DMD GENE DELETIONS

-Multiplex Amplification Using Primers Flanking Five DMD Exons

-Template DNA Prepared From Amniotic Fluid Cells

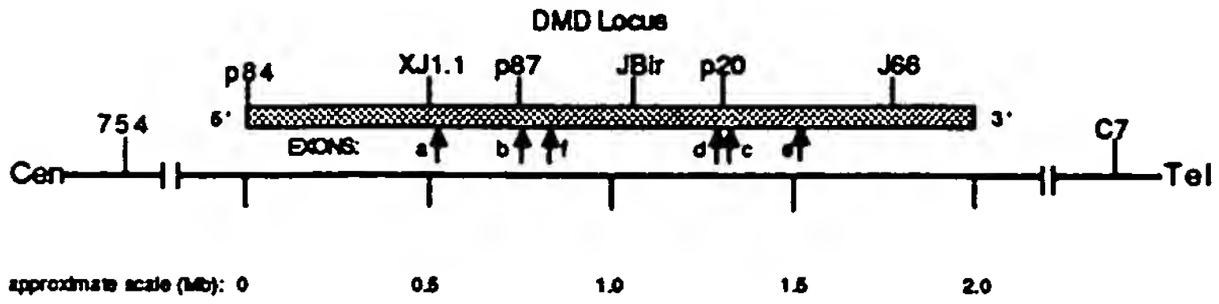


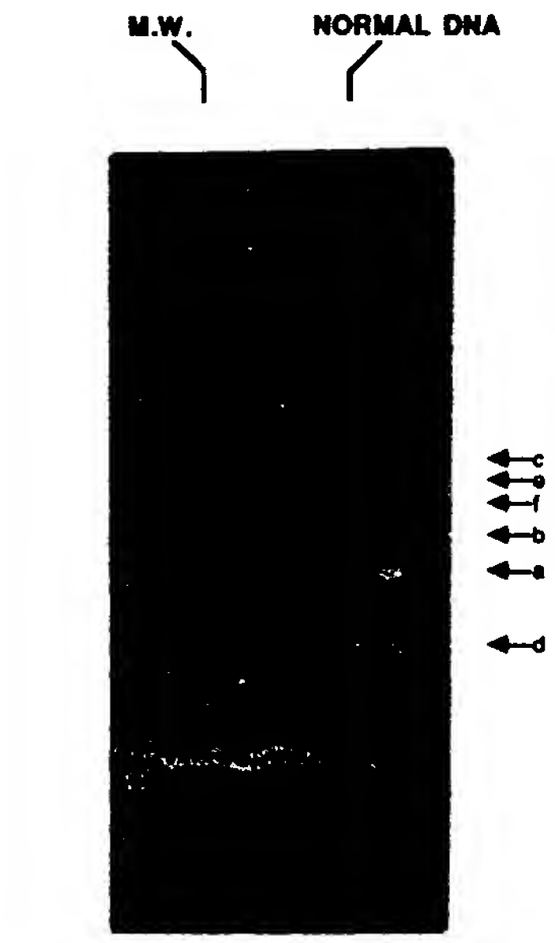
Diagnosis: DLN DLN NORM

1/18/8

MULTIPLEX DNA AMPLIFICATION AT THE DMD GENE

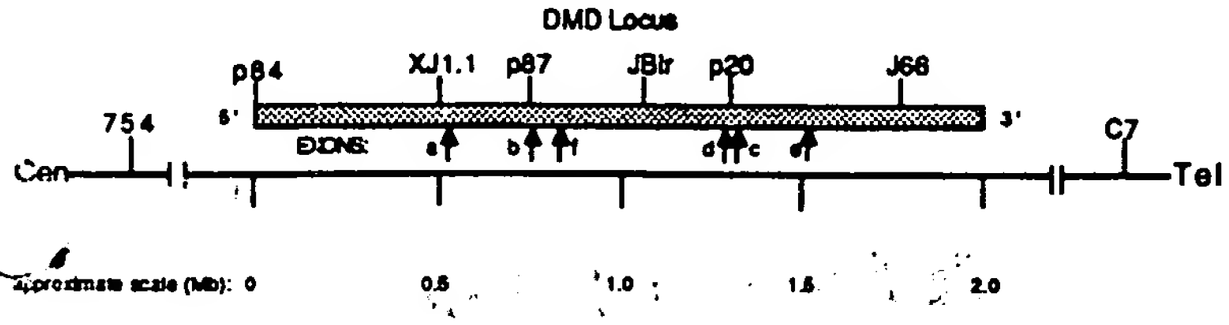
-Primer sets flanking six exons

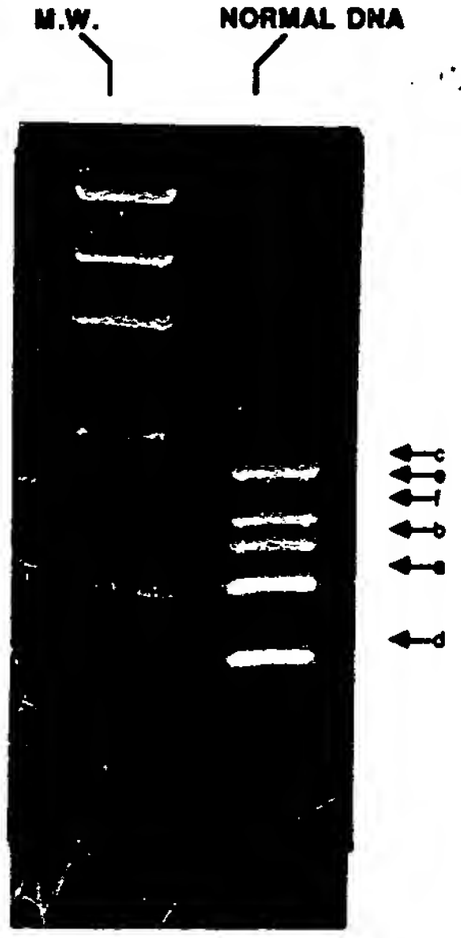




MULTIPLEX DNA AMPLIFICATION AT THE DMD GENE

-Primer sets flanking six exons





Labelling of pXD-1 for Hu. Gen. Lib.

[redacted]/87

E_1

$235896 \div 3 \times 1000 \times 2 = 1.6 \times 10^8$ cpm

F_2

$49253 \div 3 \times 950 \times 2 = 3.1 \times 10^7$ cpm

4.7×10^9 dpm/ug

T=002.00 A=235895.0(0.3%) B=235862.5(0.3%)

F_1

T=002.00 A=049250.0(0.7%) B=049244.0(0.7%)

F_2

LABELLING OF XJ10 (to screen Hu. Gen. Lib.)

[redacted]/87

F_1

$54,402 \div 3 \times 1100 \times 2 = 4.0 \times 10^7$

F_2

$17,988 \div 3 \times 600 \times 2 = 7.2 \times 10^6$

2.36×10^9 dpm/µg

7/30

Screening of Hu. Gen Lib. w/ XJ10 in progress

Washed filters → 2x SSC, 0.1% PPi, 0.1% SDS
for 2x 5 min. RT
2x 20 min 60°C
0.5x SSC (diluted 2x SSC...)
for 15 min, 45°C

On Film

Also rescreened 2° from Mouse cDNA Lib. (w/ XJ10)
Titer of pk 19.4 from Hu. Gen. Lib. vs pXL1 = 2.0×10^4/ml
Plated out ~2000 pfu (100λ of undiluted pk)

- Since transformation of DH5α w/ DMD clones was unsuccessful competent T6-1's were prepared and transformed
- Amplified 4° and plated stock (12.1 & 12.2 from Hu. Gen. Lib. vs. XD-1) Add 5ml SM, shake gently ~2hrs

12.1
titer = 1.8×10^6/ml
$50 / 1.8 \times 10^6 = .028\mu l$
$1/10^3$ dilution, take $28\mu l$

12.2
titer = 4×10^5/ml
$50 / 4 \times 10^5 = .125\mu l$
$1/10^3$ dilution, take $125\mu l$

- Washed filters from plate 19 (high density) XD-1, last wash 0.5×SSC
- replated NT.1 (from HGL vs XJ10)
- photographed gel characterizing XJ10

Titering Amplified (pure) Stock Phage [redacted] 87

from Hu. Gen. Lib. vs. pXD-1

12.2 — 1/10³, 28λ

1 - 21
2 - 37
3 - 36
4 - 49
total 143

143 x 35.7 = 5.1 x 10³/ml x 10³ = **5.1 x 10⁶/ml**

12.1 — 1/10³, 125λ

1 - 34
2 - 56
3 - 37
4 - 78
total 205

205 x 8 = 1.64 x 10³/ml x 10³ = **1.64 x 10⁶/ml**

12.2

$3 \times 10^5 \div 5.1 \times 10^6 \approx 60\,\mu l$

12.1

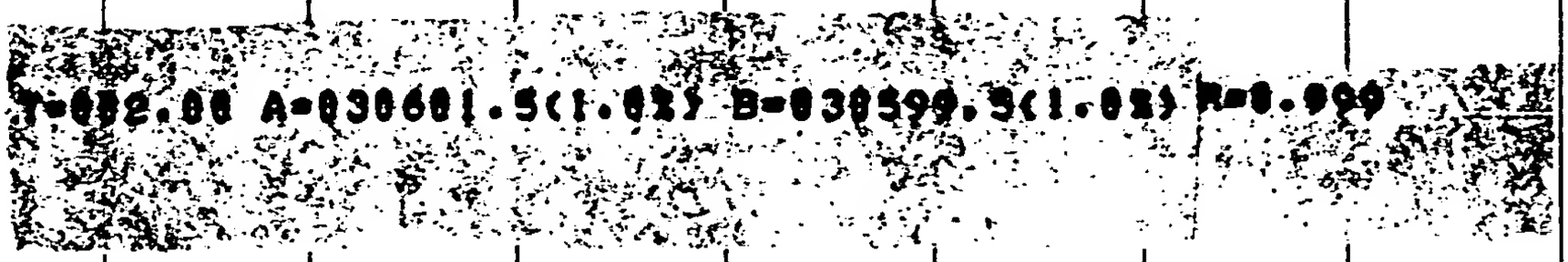
$3 \times 10^5 \div 1.64 \times 10^6 \approx 180\,\mu l$

- no positives on film w/ 19.1, 19.2, 19.4, vs. XD-1
- ~~cell~~ TG-1's transformed by DMD clones
- plated out 300,000 pfu onto lg. Amp plate
- eluted phage - 12.1, 12.2
- lifted from replating of NT.1
- made 200mls hyb. (40%F), no H.S. DNA
- made Neutralizing solution (1.5 NaCl, 0.5 Tris HCl pH 7.5

Labelling of XJ10; Isolation of DNA 12.1, 12.2 [redacted] 87

½ nan.
XJ10
F_1
30601 ÷ 3 × 1100 × 2 = 2.2×10^7 cpm
20,000 cpm/µl

F_2



Eluted 9.6mls from 1g plates w/ 12.1 and 12.2
Spun down debris and removed supernatant
Added 1ug/ml DNAse I and RNase A - 37° 30'
PEG precipitated, aspirated supernat. + to remove
Respun and removed remaining PEG w/ pipet
Resuspended in 1ml SM, tranferred to microtube
Spun to remove debris
Transfered to blue tube, diluted to 2ml w/ STE
Added ½0th vol. 10x SET (100>1), Add prok to .1mg/ml
Extracted w/ phenol, chlor/phenol, chlor.
Added 2 vol. EtOH, let sit in freezer
over night

Filters NT.1,2,3,20 on film Completed [redacted] /87

Digestion / Isolation of 12.1, .2

Spun EtOH precipitated DNA, removed supernatant
Washed w/ 70% EtOH, removed supernatant
Pooled 1st EtoH supernatant w/ TE + Am Ac + 3mls EtO
Put at 37°C
~~Add~~ Spun at 10K for 15 min.
Washed w/ 70% EtOH
Dry Vac for 20 min.

Am Ac was added along with the TE, not resuspended 1st
Heating for 5 min at 65°C to aid in resuspension
Most of DNA resuspend ed, put at 65°C for a couple more minutes
Added 3mls EtOH
37°C 10', 65°C 5'
Spun at room temp. 15'
Resuspended in 0.5ml TE
Spun briefly
Transfered to eppendorfs
Placed in Box I as phage lysates 12.1, 12.2

(12.1) 2.3 mg/ml

(12.2) 2 mg/ml

Analysis of 12.1 and 12.2 by Restriction Digest

	DNA (µl)	10x Enzyme Buffer (µl)	Enzyme (µl)	TE (µl)
1	Marker 20			
2	12.1 4.3	B*	5 BamHI 5	30.7
3	12.1 4.3	H	5 Sal I 5	30.7
4	12.1 4.3	E*	5 EcoR I 5	30.7
5	12.2 5	B*	5 BamHI 5	30
6	12.2 5	H	5 Sal I 5	30
7	12.2 5	E*	5 EcoR I 5	30
8				
9	Spermidine 2x			
10	RNase A 3x			
11				
12				
13				
14				
15				
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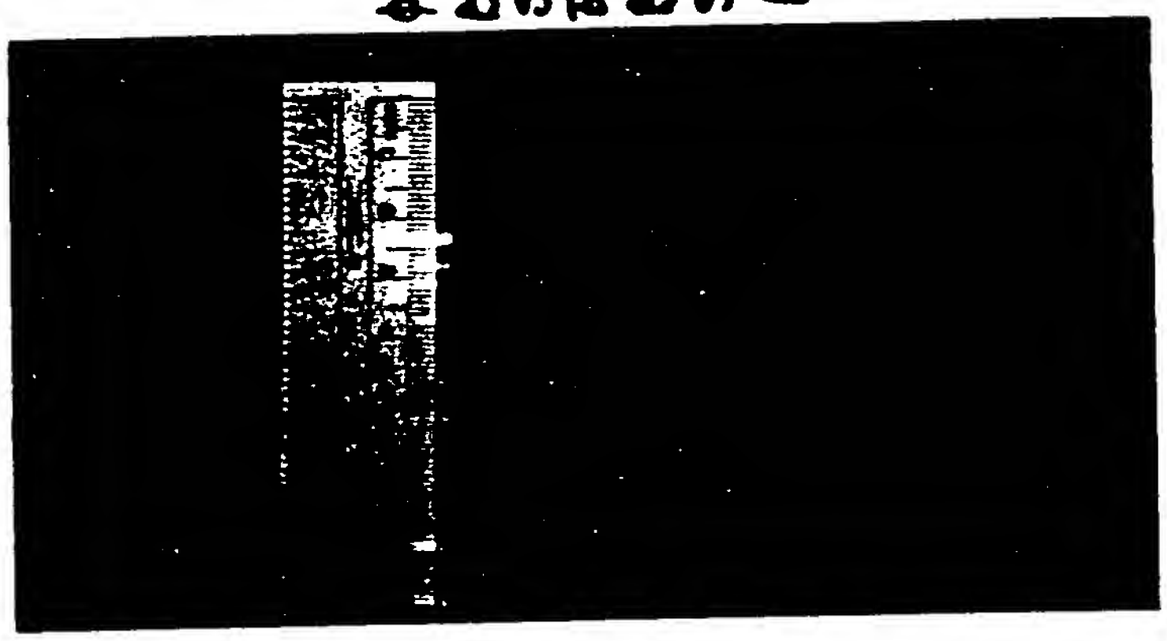

Extracting Eth. Brom. [redacted]/87

Added IsoAmyl Alcohol (5ml) to plasmid DNA taken of $CsCl_2$ density gradient
Shake vigorously for one minute
Spin at 3000 rpm for ~~5 min~~ 3 min
Repeated 3 X

DMD Clones

	1 DNA	2 (µl)	3 Buff	4 (µl)	5 Enz	6 (µl)	7 RNase A	8 Spermidine	TE	Total
1	Marker	20								20
2	30.1(3)	5	E	2	EcoRI	2	2	1	3	15
3	30.1(4)	5	E	2	"	2	2	1	3	15
4	30.2(5)	5	E	2	"	2	2	1	3	15
5	30.2(6)	5	E	2	"	2	2	1	3	15
6	44.1(7)	5	E	2	"	2	2	1	3	15
7	44.1(8)	5	E	2	"	2	2	1	3	15
8	47.4(9)	5	E	2	"	2	2	1	3	15
9	47.4(10)	5	E	2	"	2	2	1	3	15
10	63.1(11)	5	E	2	"	2	2	1	3	15
11	63.1(12)	5	E	2	"	2	2	1	3	15
12	9.7 (1)	5	2 + ½ IM NaCl	2	Hind, Eco	1, 1	2	1	2	15
13	9.7 (2)	5	2, "	2,	Hind, Eco	1, 1	2	1	2	15
14										
15	5µl dye									
16	1½ hr. digest									
17										
18										
19										
20										
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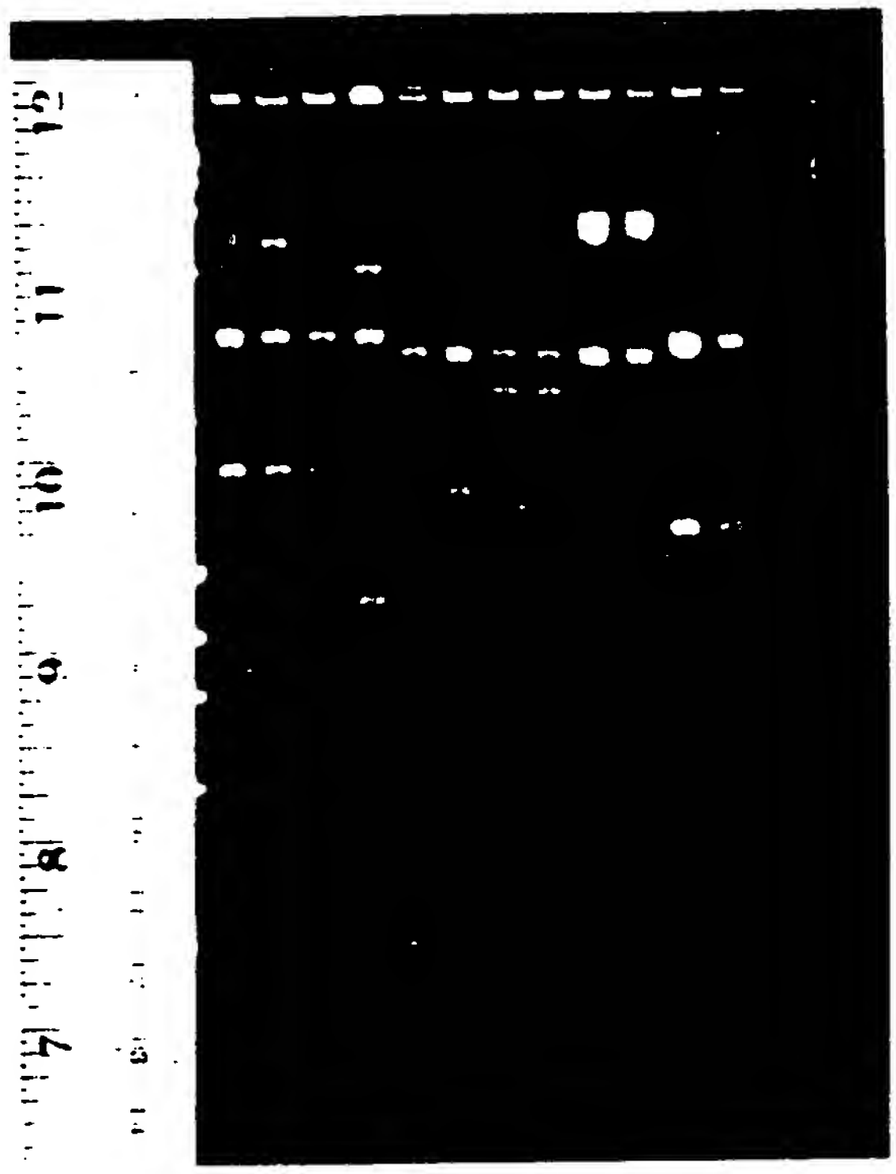


EFFICIENCY LINE • 22-206

Digest #100pm

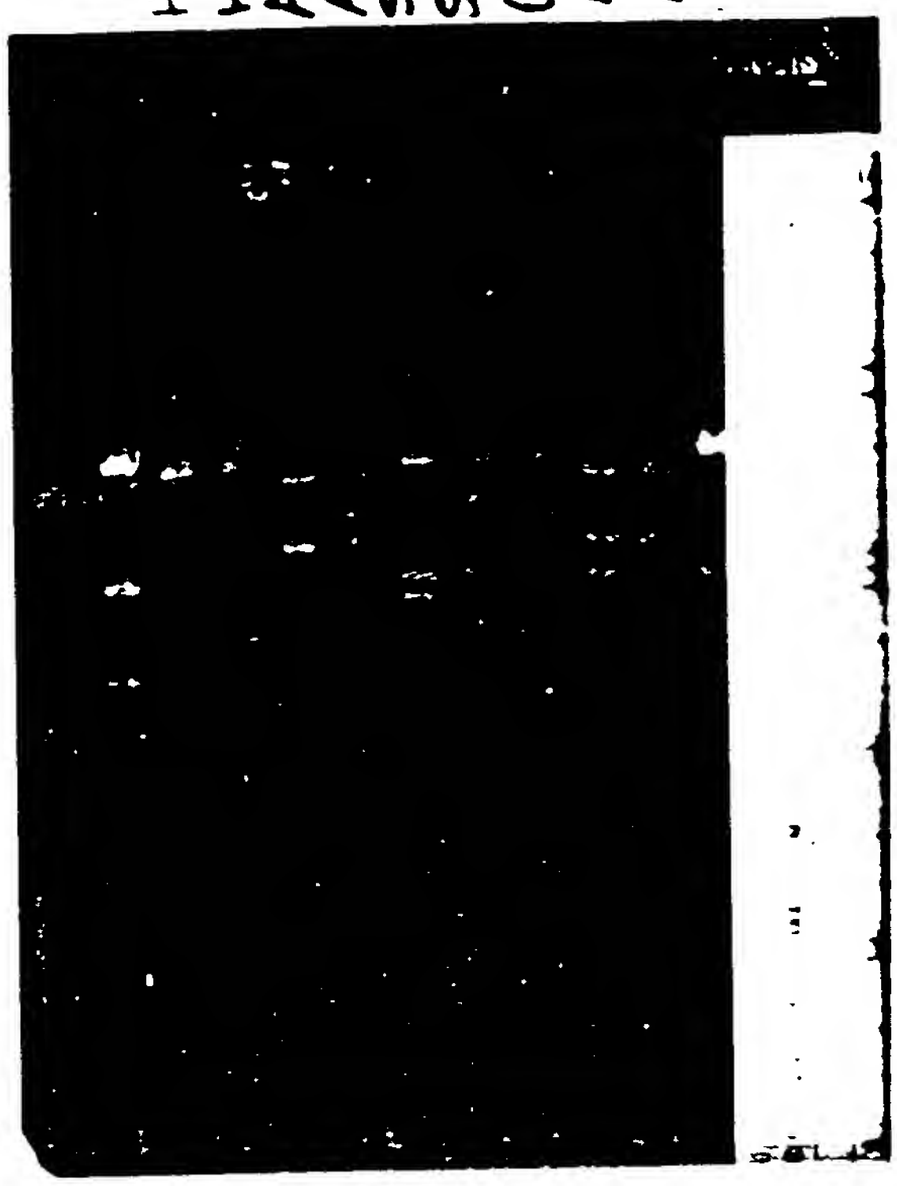
2nd Gel = 12.1, 12.2 (xD-1)

	1 DNA	2 (µl)	3 10xB	4 (µl)	5 Enz	6 (µl)	7 TE (µl)	8 Spermidine 2	RNase A 3
1	MARKER	20							
1	12.1	5	2	√5	HindIII	5	23	2	3
2	12.2	5	2	√5	HindIII	5	23	2	3
3	12.1	5	2	√5	Asp718	5	23	2	3
4	12.2	5	2	√5	Asp718	5	23	2	3
5	12.1	5	3	√5	Sal+Bam	3+2	22	2	3
6	12.2	5	3	√5	Sal+Bam	3+2	22	2	3
7	12.1	5	2,3	√5	HindIII, Sal	2+2½ (40 / 25)	21.5	2	3
8	12.2	5	2,3	√5	HindIII, Sal	2+2½ (40 / 25)	21.5	2	3
9	12.1	5	2,3	√5	Asp, Sal	2.5+2.5	21	2	3
10	12.2	5	2,3	√5	Asp, Sal	2.5+2.5	21	2	3
11									
12									

10.1 x 13.8 cm
Sal 10units/µl
Bam 15 " / "
Hind 20 " / "
Asp718 12 " / µl (14mA)
Run o/n at 25V,
Gel size 11x14

L-7R 12.1, 12.2

HindIII
HindIII
Asp718
Asp718
Sal I+BamHI
Sal I+BamHI
(12.1 Hind+Sal)
Asp718+Sal I
Asp 718+Sal I



Analysis of 12.1 and 12.2 by Restriction Digest

	1	DNA	(µl)	10x Enzyme Buffer (µl)		Enzyme (µl)		TE (µl)
1		Marker	20					
2		12.1	4.3		B*	5	BamHI 5	30.7
3		12.1	4.3		H	5	Sal I 5	30.7
4		12.1	4.3		E*	5	EcoRI 5	30.7
5		12.2	5		B*	5	BamHI 5	30
6		12.2	5		H	5	Sal I 5	30
7		12.2	5		E*	5	EcoRI 5	30
8								
9		Spermidine	2λ					
10		RNase A	3λ					
11								
12								
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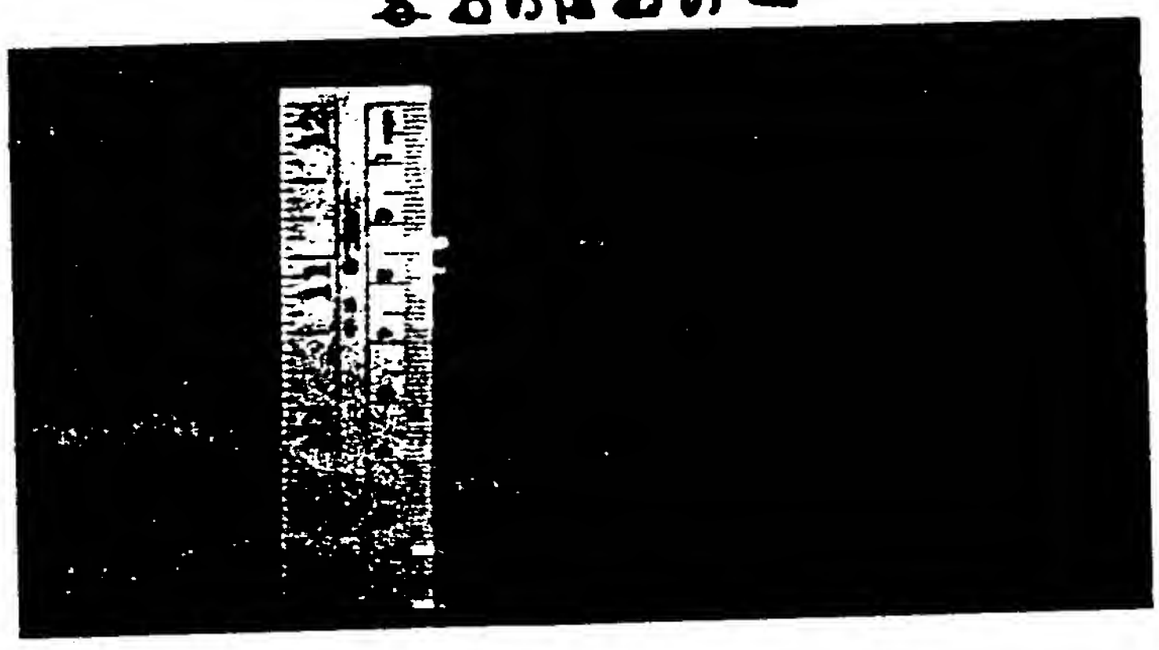
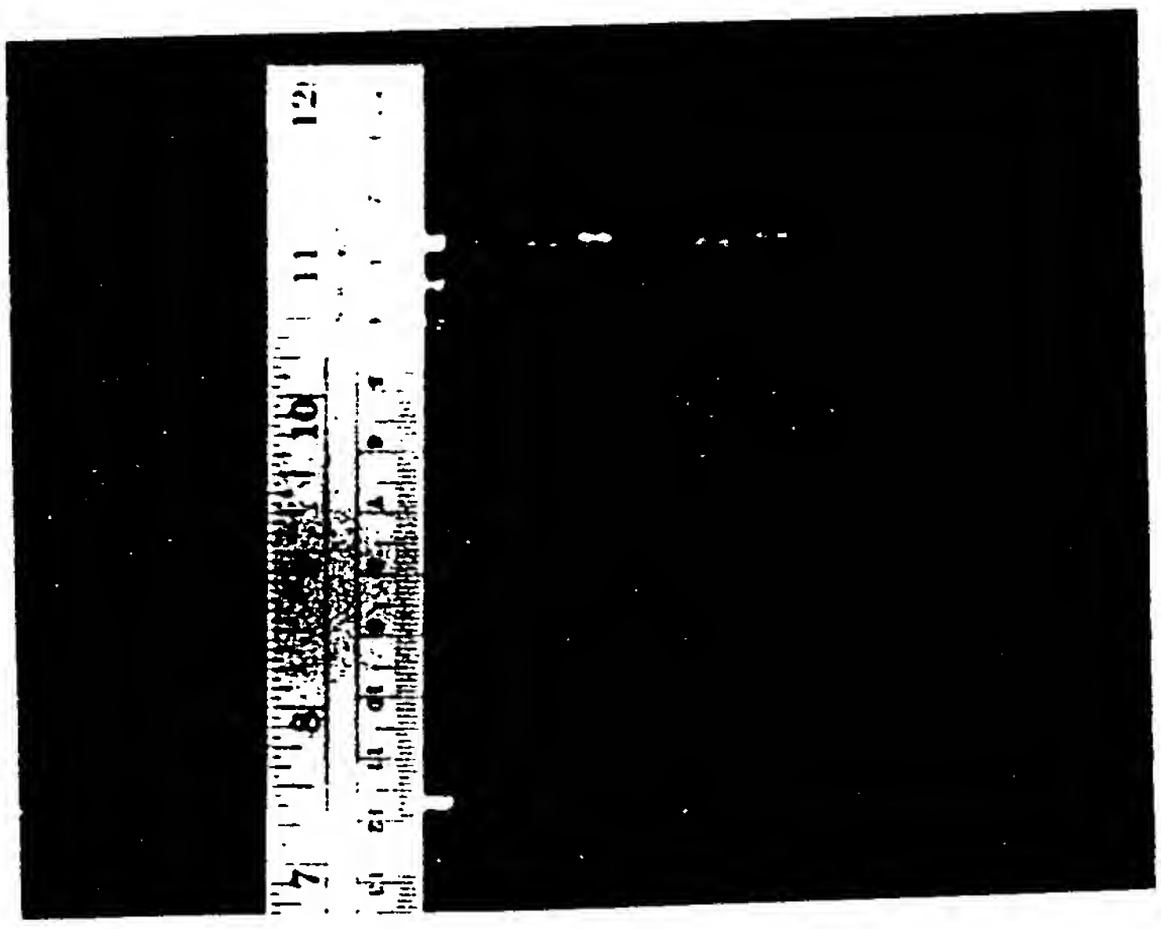




Lifts of NT.1, NT.2, NT.3, 20 (Hu Gen Lib-VS XJ10) [redacted]87

Phage eluted overnight 8/9
Picks (3°) taken from ~~3°~~, NT.1, NT.2, NT.3 (certain pos)
Replated 2° NT.1, NT.3, 20
Lifts were made from

NT.1	NT3	NT.2	20
2°, 80λ	2°, 30λ	3°, 30λ	2°, 30λ
3°, 50λ	3°, 30λ		

Needed to replate NT.1 3°, 50λ → used 10λ
Gel run on 12.1, 12.2 phage lysate
- could have some protein contamination because phenol/chloroform extraction not shaken enough

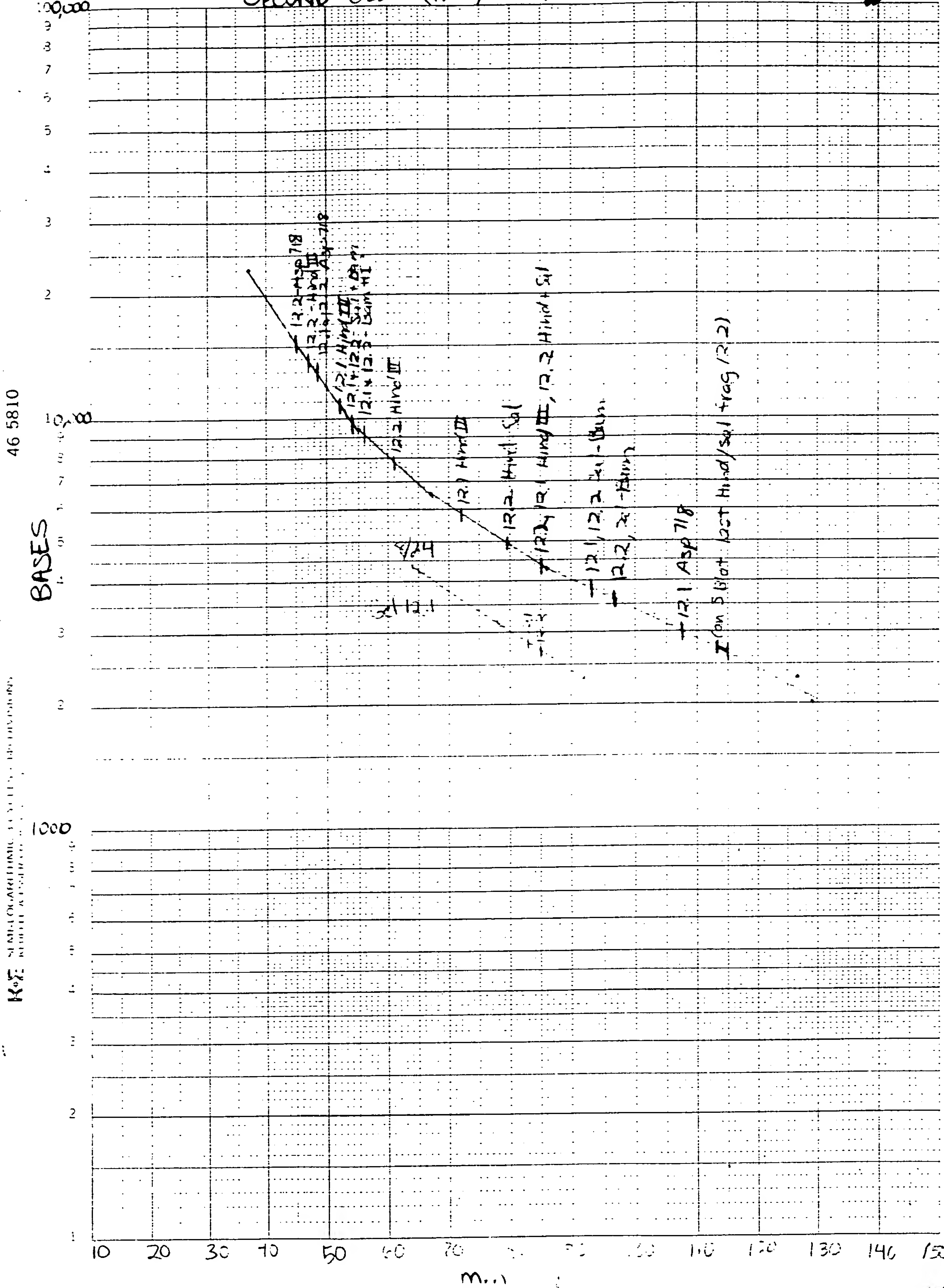
Prehyb. 6 lifts above, NT.1, NT.3, NT2, 20
Hyb. - Added 6×10^6 cpm to 6 filters
XJ10 labelling (8/6/87) 20,000cpm/μl

Fragment Sizes of second Gel 12.1, 12.2

1	2	3	4	5	6	7	8	9
12.1	12.2	12.1	12.2	12.1	12.2	12.2	12.1	12.2
HindIII	HindIII	Asp718	Asp718	Sal+Bam	Sal+Bam	Hind Sal	Asp Sal	Asp Sal
				9.2	9.2			
10.8Kb	13.8Kb		15.3Kb	9.7Kb	9.7Kb	9.2Kb	12.9Kb	12.9Kb
5.85Kb	7.8Kb	12.9Kb	12.9Kb	9.1Kb	9.1Kb	4.95Kb	9.1Kb	9.1Kb
4.35Kb	4.35Kb	3Kb	(2.85)	3.8Kb	3.8Kb	4.35Kb	(3.0)	(2.85)
					3.6Kb	(2.7)		

Library constructed w/ EMBL3 → 44 Kb
(takes up to 23Kb insert)

SECOND GEL (12.1, 12.2)



[redacted]/87

Developed Autoradiogram ī 2° and 3° of Hu. Gen. Lib.
vs. XTIO
Picked NT. 2, 3°, 30λ and NT. 3 3°, 30λ
Plated out 4° and titered - see next page
Blotted second ge 12.1 and 12.2
Washed, baked and washed first blot 12.1 and 12.2
Prehybed lift from less dense NT. 1 -10λ (3ml 40%F
and first blot 12.1 + 12.2 (7.5ml 50%F)

Titering 3° of NT.3 and NT.2

NT.2 30λ

39 x 33.33 = 1300/ml = 1.3/μl

NT.3 30λ

459 x 33.33 = 15,300/ml = 15.3/μl

*use 100λ of full strength ≅ 1500 pfu

1-78
2-57
3-69
4-116
5-69
6-80

459

Mini-Prep of DMD Clones

7

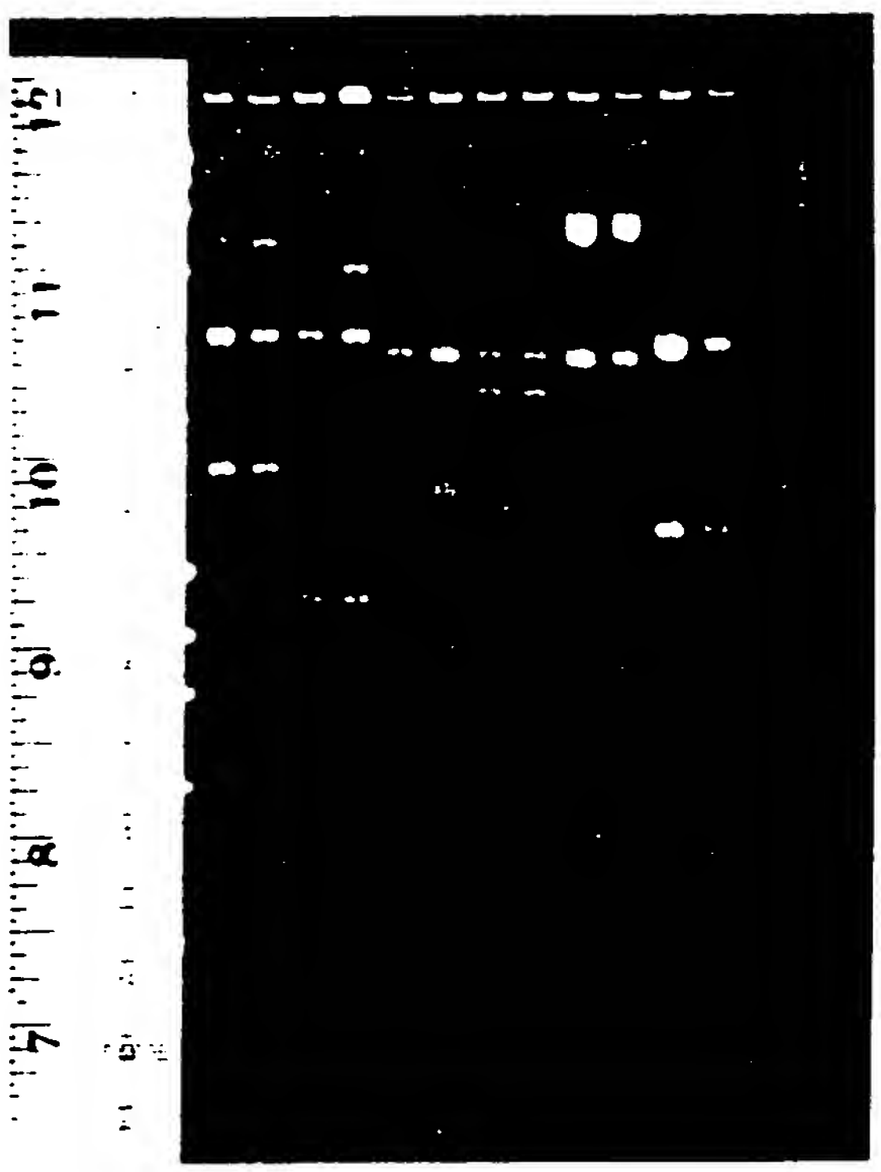
	1	2
1	9.7a	1
2	" b	2
3	30.1a	3
4	" b	4
5	30.2a	5
6	" b	6
7	44.1a	7
8	" b	8
9	47.4a	9
10	" b	10
11	63.1a	11
12	b	12

To make soln. 2 Add H_2O 1ST SDS NaOH
O/N culture, 2 picks from each clone
1 ml of gently vortexed culture into microfuge
Spun down bugs 15 sec
Aspirated supernatant
Resuspended in 100µl soln I by vortexing vigorously
Added 200µl soln. 2, inverted 6x ice 5 min
Added 150µl soln 3, inverted 3x, ice 30 min
Vortex gently
Spun 4 min
Poured sup. into tubes containing 800µl EtOH each
-70, 15' (layer weren't well mixed, thawed, mixed, -70, 10'
Spun 4 min

DMD Clones

[redacted]/87

	1 DNA	2 (µl)	10x Buff	3 (µl)	5 Enz	6 (µl)	7 RNase A	8 Spermidine	TE	Tot
1	Marker	20								20
2	30.1(3)	5	E	2	EcoRI	2	2	1	3	15
3	30.1(4)	5	E	2	"	2	2	1	3	15
4	30.2(5)	5	E	2	"	2	2	1	3	15
5	30.2(6)	5	E	2	"	2	2	1	3	15
6	44.1(7)	5	E	2	"	2	2	1	3	15
7	44.1(8)	5	E	2	"	2	2	1	3	15
8	47.4(9)	5	E	2	"	2	2	1	3	15
9	47.4(10)	5	E	2	"	2	2	1	3	15
10	63.1(11)	5	E	2	"	2	2	1	3	15
11	63.1(12)	5	E	2	"	2	2	1	3	15
12	9.7 (1)	5	2 + 1/2 1M NaCl	2	Hind, Eco	1, 1	2	1	2	15
13	9.7 (2)	5	2, "	2,	Hind, Eco	1, 1	2	1	2	15
14										
15	5µl dye									
16	1½ hr. digest									
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										



EFFICIENCY LINE • 22-206

Finished mini-prep of DMD cDNA
For hyb. of NT.1, 10λ used 5ml probe (XJ10), 60ml H.S.DN
Ran gel of DMD cDNA
Prehybed Second gel of 12.1, 12.2
Both in refrig. over the weekend (First + Second gel of 12.1, 12.2)
Start 41088 culture

For the weekend

Titer stock
Wash and expose NT.1 vs. XJ10
Size DNA fragments from
1) Characterization of XJ10
2) First gel 12.1, 12.2
3) Second gel 12.1, 12.2

Estimation of Amplified phage lysate titer

From previous plates titers were determined

NT.2 and NT.3

NT.2 1.25×10^4/ml (2500 on plate to elute) ÷ 5ml (SM) = 6.25×10^6/ml

NT.3 1.5×10^4/ml (1500 ") ÷ 5ml " = 4.5×10^6/ml

NT.2 $50 \div 6.25 \times 10^2 = 80\mu l$

NT.3 $50 \div 4.5 \times 10^2 = 110\mu l$

Plated out NT.2, $1/10^3$, 80λ
$1/10^4$, 80λ
NT.3, $1/10^3$, 110λ
$1/10^4$, 110λ

Titer of NT.2, NT.3

NT.3	110λ, $1/10^4$
1-42	
2-55	$329 \times 9.1 \times 10^4 =$ **3×10^7/ml**
3-64	3λ
4-73	Plate out ~~10λ~~ of full strength
5-95	for 400,000
329	

NT.2 $1/10^4$, 80λ

1500

$1500 \times 12.5 \times 10^4 =$ **1.9×10^8/ml**

~~$1/10$ (10λ, 90λ) dil., 15.8μl~~ 2.1λ

4/15/87

Started Lg. scale plasmid prep. for DMD cDNA clones
Prepared TB - 500ml per flask
Added Amp to 50µg/ml
Added 2mls to each
ON Shaker O/N

Picked from original 2° plates - NT. 1 B, 20

Titered amplified phage lysate for lg. scale isolation of the DNA.

Hybridization to 2 Southern Blots 12.1, 12.2

XD-1

- labelled to 65,000 counts/μl
- want 500,000 cpm/ml
- 7 ml. for Blot 1 → 54 μl
- 7.5 ml. for Blot 2 → 62 μl

116 μl

H.S. DNA → 140 μl
" → 150 μl

- Started prep of phage lysate DNA NT.2 and NT.3
- Maxi prep of DMD clones spun o/N on $CsCl_2$ gradient

- phage lysate DNA - NT.2 O.D$_{260}$ = 0.39
NT.3 " = 0.35

NT.2 → 0.39 x 100 x 50 = 1.95 mg/ml
NT 3 → 0.35 x 100 x 50 = 1.75 mg/ml

For 10µg DNA

NT.2 → $\frac{10}{1950}$ = 5.1µl ~ 5µl

NT.3 → $\frac{10}{1750}$ = 5.7µl ~ 6µl

Washed Southerns on 12.1 + 12.2
2x5' 2xSSC RT
1x30' 2xSSC 65°C
1x30' 0.1xSSC 50°C

ON Film

Set up digestion of NT.2, NT.3
0.7% Gel
Hind III
EcoR I
Sal I
Eco, Sal
Hind, Sal

Hybridization - NT.1, 20 vs XJ10

[redacted]/87

Aprox. 2 weeks ago XJ10 → 20,000 counts/μl
now → 10,000 c/μl
Want 2×10^6 counts → take 200μl
Total vol. 5 ml.

$5x = (.1)5$
$x = .1$ ml HS DNA

Results seem inconclusive
Much of the probe was lost because the sealer would not seal
Washes - 2x, 2x SSC RT 5min
2x, 2x SSC 60°C 20'
1x, .1 x SSC 45°C 15'

Set up Southern Blot of NT.2, NT.3

Completed ~~Mini~~ phage lysate DNA purification resuspended in 1 ml

DNA removed from $CsCl_2$ gradient
EtBr removed by extraction ¢ IsoAmyl Alcohol
Added 2 vol. EtOH
Freezer (-20°C) O/N

Ran gel on NT.2, NT.3

Developed Autorad. of S Blots of 12.1, 12.2
[Washes were 1x 30' 2XSSC, 1x 30' 0.1xSSC]
65°C 50°C

NH4 Ac Precipitation to Further Purify [redacted]/87

1 2 DMD cDNA and 12.1, 12.2 6 7 8 9

DMD cDNA (in 95% EtOH)
- removed from -20°C, centrifuged
- $CsCl_2$ present
- Washed w/ 70% EtOH
- Resuspended in 1 ml TE
- Heated at 37°C until $CsCl_2$ in soln.
- Transfered to new tube
- Added 50 µl 5M NaCl (vor.)
- Added .5 ml 7.5 M NH_4Ac (vor.)
- 3 ml 95% EtOH - ice 30'
- Spin 12K 10'
- 2 ml 70% EtOH
- 0.5 TE in microfuge tube

12.1 12.2 (in 0.5 ml TE)
- Add ½ Vol. 7.5M NH_4Ac (vor.) 250 µl
- Ice ~~30~~ 5'
- Microfuge 10'
- Transfer to blue tubes 1.5 ml EtOH
- 30' ice
- Spin 12K 10'
- Rinse 70% EtOH
- Resuspend 0.5 ml TE

Washed S. Blot
2x NaOH 10'
2x Neut. 10'
~~Rinsed~~ filter (hydrated) ∈ H_2O, 20x SSC
Transferred in 20x SSC
10.1 x 13.9 cm

12.2 Preparatory Gel

[redacted]/17

	1 DNA	2 (µl)	3 10xB	4 (µl)	5 Enz	6 (µl)	H_2O (µl)	8 Spermidine	RNase A
1	λ-ØX	30							
2	B12.2	250	2	40	HindIII	20	50	20	20
3				+4.4λ	+SalI	25			
4				5M NaCl	(15u/λ)				
5									
6	0.7%	Gel							
7	vol.								
8	2.5 hrs. then 2nd Enz. 2 hrs								
9	NaCl?								
10	Enz.								
11									
12	This gel sample was run on lg. gel								
13	with DMD cDNA inserts								
14									
15									
16	Small 12.2 fragment cut ∈ Hind, Sal seems								
17	to be								
18									
19	2.75 Kb								
20									
21	picture w/ Gel picture next page								
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Conc. of DNA's

	1	2	3	4	5	6	7	8	9
1		3/500 dilution ↓							
2	DNA	260	260/280	conc.	vol.	yield			
3	9.7	.479	2.00	4.0 ng/λ	½ml	2mg			
4	30.1	.433	2.00	3.6 ng/λ		1.8mg			
5	30.2	.631	1.97	5.3 ng/λ		2.6mg			
6	44.1	.325	1.98	2.7 ng/λ		1.35mg			
7	47.4	.290	1.99	2.4 ng/λ		1.2mg			
8	63.1	.182	2.00	1.5 ng/λ		760mg			
9	12.1	.171	2.28	1.4 ng/λ		700mg			
10	12.2	.132	2.30	1.1 ng/λ	↓	550mg			
11				4ng/λ					
12	12.2i			(9λ remaining)					
13	9.7i			20ng/λ					
14	30.1i			20ng/λ					
15	30.2i			20ng/λ					
16	44.1i			23ng/λ					
17	47.4i			8ng/λ					
18	63.1i			15ng/λ					
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

12.2 Preparatory Gel

	1 DNA	2 (µl)	3 10xB	4 (µl)	5 ENZ	6 (µl)	H_2O (µl)	Spermidine	RNaseA
1	λ-ØX	30							
2	B12.2	250	2	40	HindIII	20	50	20	20
3				+44λ	+SalI	25			
4				5M NaCl	(15u/λ)				
5									
6	0.7%	Gel							
7	vol.								
8	2.5 hrs. then 2nd Enz. 2 hrs								
9	NaCl?								
10	Enz.								
11									
12	This gel sample was run on lg. gel								
13	with DMD cDNA inserts								
14									
15									
16	Small 12.2 fragment cut ε Hind, Sal seems								
17	to be								
18									
19	2.75 Kb								
20									
21	picture w/ Gel picture next page								
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Gel ε 12.1, 12.2 (3rd gel) to see if 2.75 frags HindIII frag)

	DNA	(µl)	10xB	(µl)	Enz	(µl)	TE(µl)	Spermidine	RNAse A (µl)
2	12.1	7	2	5	HindIII	5	28	2	3
3	12.2	9	2	5	HindIII	5	26	2	3
4	12.1	7	2 1M NaCl	5,2.4	Hind,Sal	2,3	28	2	3
5	12.2	9	2, 1M NaCl	5,2.4	Hind,Sal	2,3	26	2	3
1	λ-PX	20							
6									
7									
8									
9									
10									

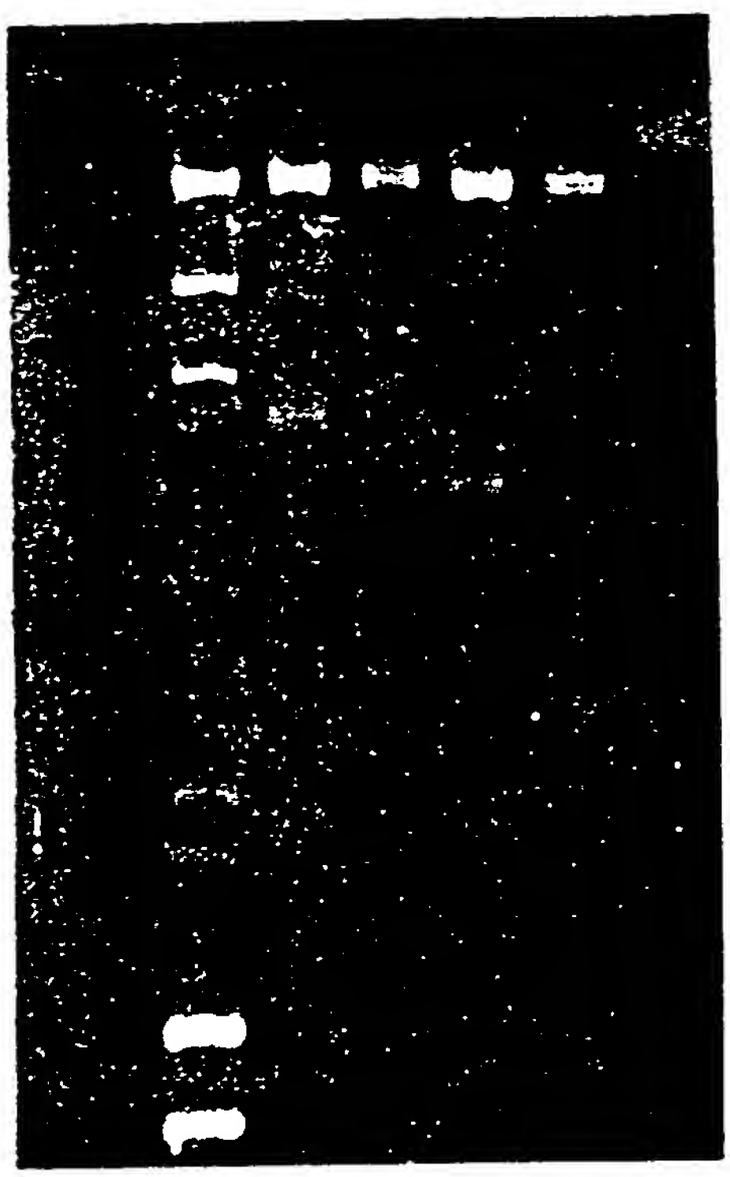
For double digests - added 2nd Enz. after 2½ hrs. then 2 hrs.

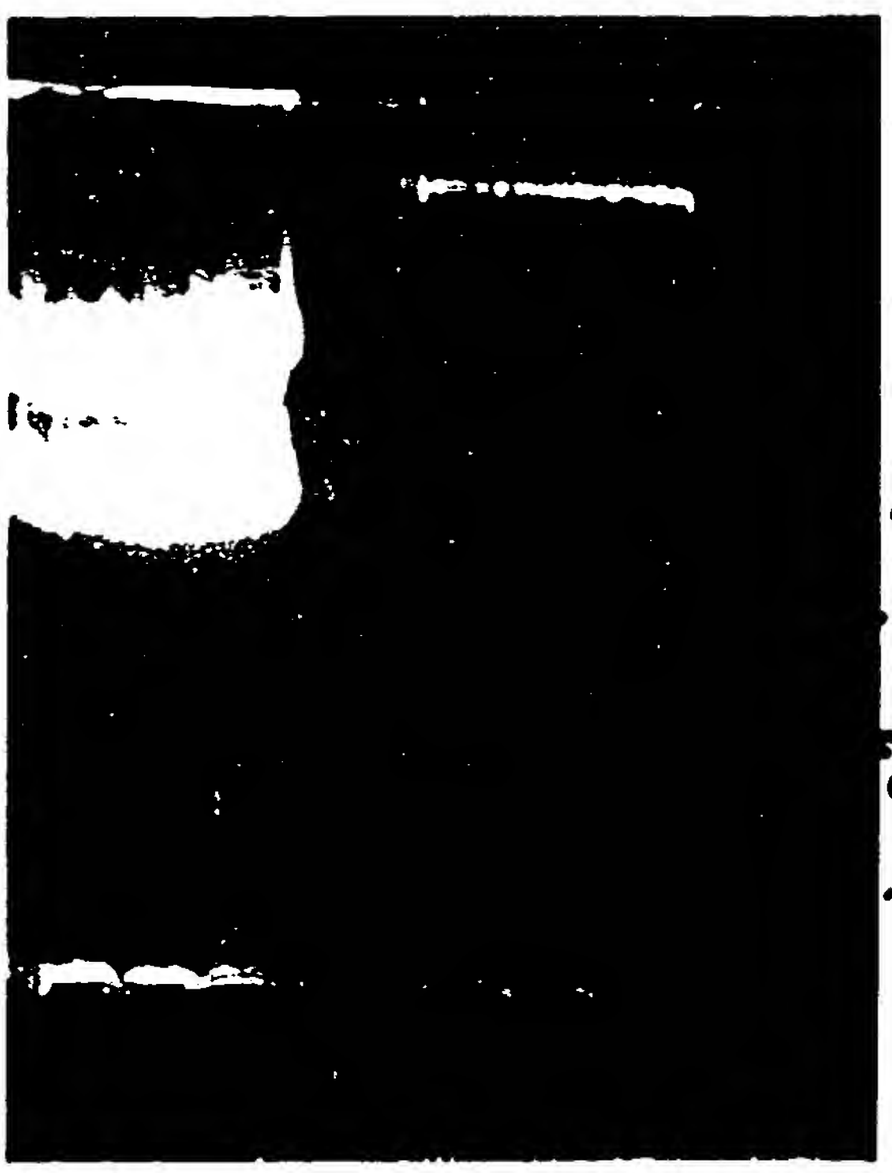
new conc.

12.1 1.4 mg/ml

12.2 1.1 mg/ml

From Prep Gel 12.2





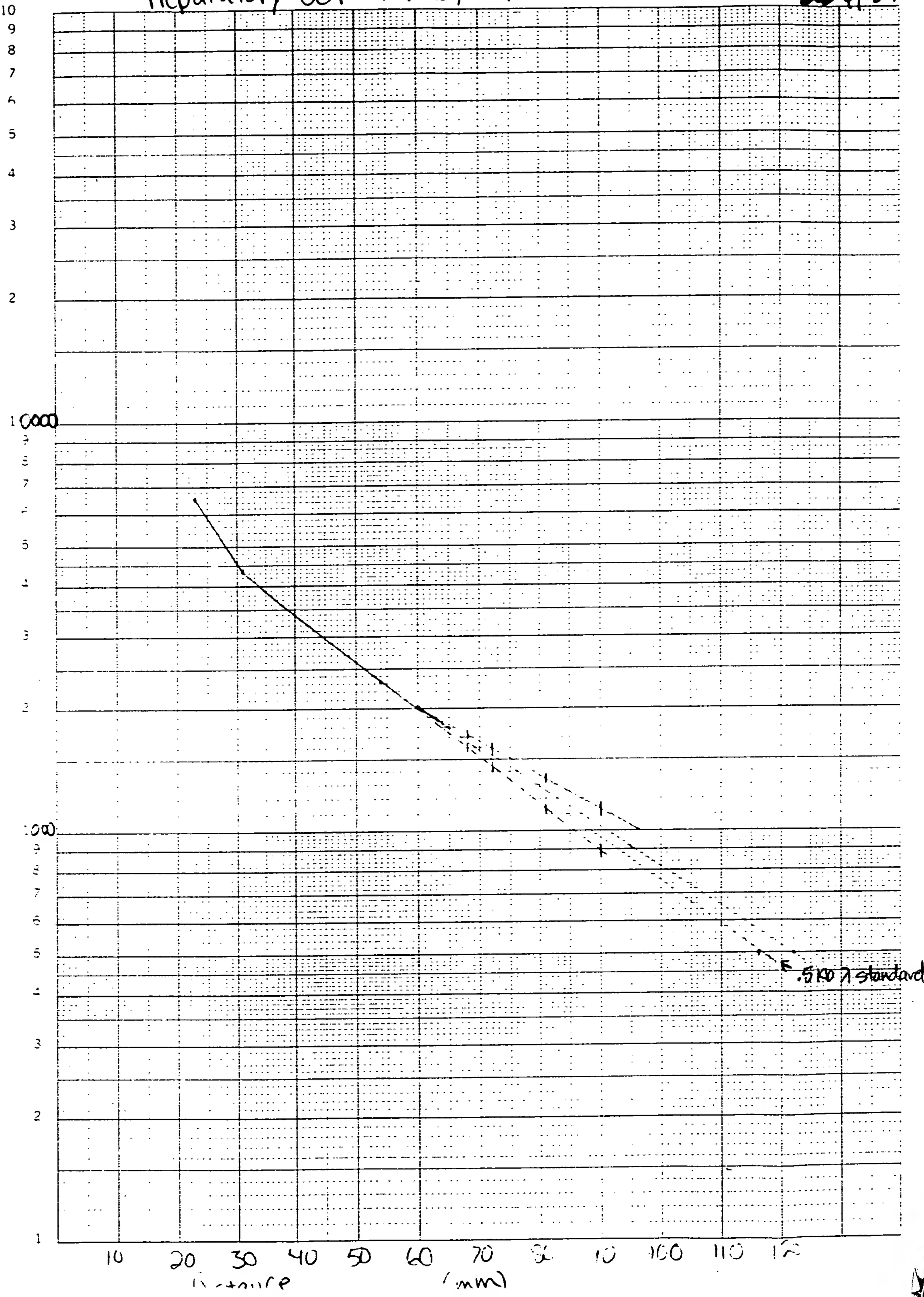
Sized quickly on graph 8/11

EFFICIENCY LINE • 22-206

ISOLATED DMD INSERTS

Clone	Size	RE Sites	Concentration	Volume
12.2	2.75kb	HindIII/Sal I	4ng/µl	10µl
NT.1b	4.30kb	HindIII	18ng/µl	10µl
NT.2	3.35kb	HindIII/Sal I	30ng/µl	10µl
NT.3	3.20kb	EcoRI/Sal I	18ng/µl	10µl
20	1.25kb	HindIII/Sal I	8ng/µl	10µl
PTZ18R	2.90kb	Sal I	50ng/µl	48µl
PTZ19R	2.90kb	HindIII	50ng/µl	80µl
47.4	0.60kb	EcoRI/Bgl II	20ng/µl	30µl
63.1	1.00kb	HindIII	40ng/µl	30µl

Preparatory Gel NT.16, 20, PT218R, PT219R [redacted]/87



Approx. Sizes of lower Bands 20 (H3/Sal) 4/4/87

	1	2	3 Range	5	Average	8	9
1	1		1.6-1.72Kb		1.66Kb		
2			1.45-1.6Kb		1.52Kb		
3	2		↓				
4							
5	3		1.13-1.35Kb		1.24Kb ←	to electrolute	
6							
7	4		0.89-1.14Kb		1.02Kb		
8							
9							
10	Fragment on gel of NT.1b and 20 at 128 mm in lane						
11	containing clone 20 is						
12					1.25Kb		
13							
14	Saved						
15							
16	20 H3/Sal frags.				1.66Kb		
17					1.52Kb		
18					1.02Kb		
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							

AMPAD · EFFICIENCY LINE · 22-206

For prep gel NT. 2, 3, 1, 4

NT 2 Hi-Eco-5+ (
3.3kb (3.35) ✓
1.8kb
(4.3-4.5 & 6.6kb)

N.T. 3 Eco-5a (
5.5kb
3.2kb ✓
2.4kb
2.2kb
1.75kb

18
22
26
35
54
63
82

39
53
65
83

3.3kb
1.8kb

10 20 30 40 50 60 70 80 90 100

KbΣ

46 5810

14 days / 2 (double) = 11 days / X

X = 1.57 (x 133% if probe fresh)

probe to be used = 210%

H.S. DNA = 140%

Recounted probe

F_1 2.9×10^5 cpm 2.9×10^3/λ

F_2 2.3×10^6 cpm 2.3×10^3/λ

Take 260λ of F_2 for 6×10^6 counts

Combined remaining probe

Added all

F_1 122 T=002.00 A=026282.0(1.0%) B=001103.5(5.0%) C=000000.0(>20%) S=0.166

F_2 122 T=002.00 A=024575.5(1.0%) B=003521.0(3.0%) C=000000.0(>20%) S=0.166

[redacted]/87

	1	2	3	4	5	6	7	8	9
1									
2			A_{260}	conc.		vol.	yield		
3	NT.1B		0.233	2.33mg/ml		0.5ml	1.17mg		
4	NT.2		0.382	3.82mg/ml		"	1.91mg		
5	NT.3		0.329	3.29mg/ml		"	1.64mg		
6	20		0.230	2.30mg/ml		"	1.15mg		
7			.147						
8									
9	For 30µg of ~~NT.2~~ → ~~8µl~~								
10	~~NT.3~~ → ~~9µl~~								
11	63.1 → 20µl								
12	47.4 → 12µl								
13									
14									
15									
16									
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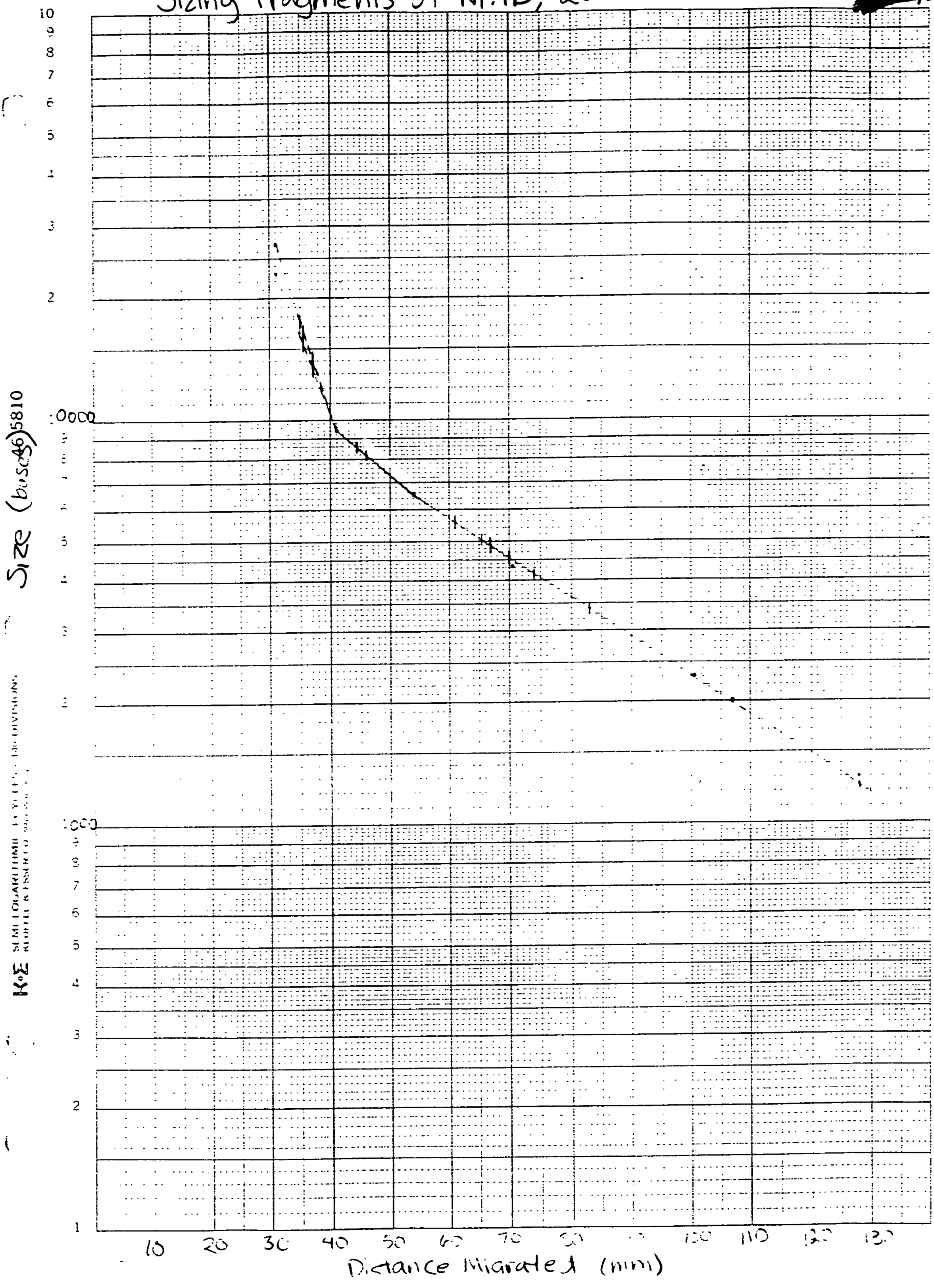
31									

Sizing Fragments of NT.1B, 20

[redacted] 87



(con't)

[redacted]/87 [redacted]/87

	DNA	LANE	3	DISTANCE MIGRATED		7	8 Size	9
1	Enzyme							
2								
3	NT.1B	9		46.3			8.20	
4	Hind/Sal			61.0			5.60	
5				65.0			5.10	
6				69.5-	70.0		4.55	
7								
8	20	10		37.0		~	13.80	
9	Hind/Sal			61.0			5.60	
10				65.0			5.10	
11				128.0			1.25	
12								
13	STANDARD							
14	3			54.0			6.55	
15	2			41.0			9.41	
16	4			70.5			4.36	
17	5			100.5			2.32	
18	6			107.0			2.02	
19	1-ϕx			128.0			1.35	
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								

EFFICIENCY LINE = 22.206

Purification of DMD clones NT.1B, 20 [redacted]/87

- 14ml. eluted from 150mm plates
- Blue tubes leaked a little when phenol/chlor. extracting
- Left in 95% EtOH O/N
- 15' -70°C
- Cfg 15' 11K, 4°C
- Wash 70% EtOH, Tubes collapsed- no leakage
- Dry
- Resuspend 0.5 ml

9/1/87

NT.1B 5λ → 0.5ml

OD_{260} = 0.38 x

0.38 x 200 ÷ 20 = 3.8 µg/µl

30 µg ≅ 8.0 µl

20 5λ → 0.5ml

OD_{260} = 0.35

0.35 x 200 ÷ 20 = 3.5 µg/µl

30 µg ≅ 8.5 µl

NT.1B 1/10⁴, 3.6λ

1-54
2-31
3-36
4-33
154

154 × 277.78 = 4.2 × 10⁴ × 10⁴ = 4.2 × 10⁸/ml

20 1/10⁴, 10↗

2.000 × 100 × 10⁴ = 2.0 × 10⁹/ml

NT.1B - 400,000 ÷ 4.2 × 10⁸ = 1/ml
Do 1/10 dil. take 10 µl

20 - 400,000 ÷ 2.0 × 10⁹ 20
Do 1/100 dil. take 10.7 µl

EFFICIENCY LINE 22-206

Screening NT.1B, 20 4° Lifts

[redacted] 4/7

- XJ10 labelled to 45,000 counts/μl (F1 labelling 8/24)
- 5ml at 1×10^6 counts/ml
- $\frac{5 \times 10^6}{4.5 \times 10^4}$ = 110 μl
- For 0.1mg/ml HS DNA
  5x = (0.1)5ml
  x = 100λ

Titering NT.1B, 20 Amplified phage lysate

From 8/27 titers were NT.1B - 84.2 pfu/μl, 8.4×10^4/m
20 - 51 pfu/μl, 5.1×10^4/m

NT.1B 100λ

(8400 x 10 =) 8.4×10^4/ml
8.4×10^4/ml (8400 on plate) ÷ 5ml = 1.4×10^8/ml

20 100λ

5.1×10^4/ml (5100) ÷ 5 = 5.2×10^7/ml

50 ÷ 1.4×10^3 = 36μl NT.1B
50 ÷ 5.2×10^3 = 9.6μl 20

20

- 1/100 → 5.2×10^5 ~~10λ~~
  1/100 → 5.2×10^3 → ~~10λ~~, 100λ

NT.1B

1/100 → 1.4×10^6
1/100 → 1.4×10^4 → 3.6, 36λ

Titering 20 and NT.1B For Amplification [redacted]

20

5λ 4°

1-90
2-52
3-48
4-65

255 x 200 = 51/μl
1.5x10³/51 = 30λ of a pick from 4° plate

NT.1B 5λ 4°

1-45
2-162
3-65
4-99
421

421 x 200 = 84.2/μl
1.5x10³/84.2 = 20λ of a pick from 4° plate

Ethanol Precip. of Electroluted DMD cDNA [redacted]

- Samples removed from -20°C
- Placed 15-30' at -70°C
  " 37°C 5' (to get agar from electrolution heated)
- Centrifuged horizontal microfg. 12'
  " angle " 10'
- Removed supernatant carefully
- Added 200λ 70% ethanol
- Centrifuged in angle in cold room 5'
- Removed supernatant
- Speed vac 5'
- dissolved in 50λ TE
- ~~R~~

Washing Filters NT.1 .1B NT.20, NT.3 [redacted]/7

For small filters:

2X	2xSSC	RT	5'
2X	2xSSC	65°C	20'
1X	0.1xSSC	45°C	~~20~~ 15'

For S. Blot of NT.2, NT.3

2X	2xSSC	5'	RT	
1X	2xSSC	30'	65°C	
1X	0.1xSSC	30'	45°C	- better at 50°C (less background Comparing S Blot 12/12. to results from here

Results of Autorads

8/26/7

- 3 Picks from NT.1B, 20 appear positive
- Will pick again and titer tonight

- Even though S. Blot autorad. went O/N looks good
  no internal HindIII sites

NT.2 and NT.3 from Human Genomic Library vs. XJ10

46 5810

K+E SEMI-LOGARITHMIC 1 CYCLE
KEUFFEL & ESSER CO. MADE IN U.S.A.

Bases

100 000
9
8
7
6
5
4
3
2
10 000
9
8
7
6
5
4
3
2
1000
9
8
7
6
5
4
3
2
1

?

10 20 30 40 50 60 70 80 90 100 110 120 130

min

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	[1]DNA	[2]Lane	[3] Distance migrated				Size	Fragment	
1	NT. 2	7	530	mm			9.41	Kb - λ	
2	Eco, Sal		√55.5	mm		13.4	8.70	Kb	
3			~~55.5~~ 78.0	mm			4.70	Kb - λ	?
4			82.0	mm			4.36	Kb - λ	?
5	NT. 2	8	~~92.0~~ 53.0	mm			9.41	Kb - λ	
6	Eco, Sal		√56.0	mm			8.50	Kb	
7			√74.0	mm			5.10	Kb	
8			49.0?	mm					
			√43.0				17.50	Kb	
			√49.0				14.50	Kb	
9	NT 2	9	49.0	mm			11.00	Kb	
10	Hind, Sal		53.0	mm			9.41	Kb - λ	
11			78.0	mm			4.70	Kb - λ	
12			82.0	mm			4.36	Kb - λ	
13			92.0	(faint)? mm			3.63	Kb -	
14	NT. 3	10	49.0				11.00	Kb	
15	Hind, Sal		53.0				9.41	Kb - λ	
16			√74.0?				5.10	Kb	
17			78.0				4.70	Kb - λ	
18			82.0				4.36	Kb - λ	
19									
20	NT. 2	9	√105.0				2.95	Kb	
21	(autorads)								
22	NT. 2	9	√110.0				2.70	Kb	
23	NT. 3	10	√91.0				3.95	Kb	*
24									
25									
26									
27									
28									
29									
30									
31									

Gel Distances NT.2, NT.3

[redacted] 17

λ-HindIII	Distance migrated	Size fragment
1	38 mm	23.13 Kb
2	52 mm	9.41 Kb
3	63.5 mm	6.55 Kb
4	82 mm	4.36 Kb
5?	~420 mm	2.32 Kb

DNA	Lane	Distance migrated	Size fragment
NT.2	1	48.5 mm	12.30 Kb
HindIII		63.5 mm	6.55 Kb - λ
		75.0 mm	5.0 Kb
		82.0 mm	4.36 Kb - λ
NT.3	2	✓45.0 mm	15.30 Kb
HindIII		✓51.5 mm	10.40 Kb
		✓55.0 mm	8.80 Kb
		77.0 mm	4.80 Kb
		82.0 mm	4.36 Kb - λ
NT.2	3	51.0 mm	10.60 Kb
EcoRI		82.0 mm	4.36 Kb - λ
		91.5 mm	3.68 Kb
NT.3	4	✓46.0 mm	14.5 Kb
EcoRI		56.0 mm	8.5 Kb
		90.0 mm	3.75 Kb
NT.2	5	✓42.0 mm	18.5 Kb
SalI		52.0 mm	10.00 Kb
NT.3	6	48.0 mm	12.80 Kb
SalI		530 mm	9.41 Kb - λ
		✓74.0 mm	5.1 Kb

Electroelution of DMD cDNA Inserts

Let slices sit in H_2O, (15-1.5ml) - inverting every 5' or so (to rel sal
Set up cups w/ Dialysis Membrane
Filled w/ H_2O to check for leaks
Emptied, decanted H_2O from orange capped tubes (15m
Removed slices, blotted dry ~~c~~ Kimwipe
Cut into small cubes on parafilm, wiping razor after each sample
Pieces placed in lg. well - loaded onto electroelution box
Allowed to elute for ~2hrs.

Jeff removed DNA.

Hybridization of XJ10 to NT.1, NT.3, 20

[redacted]

$\frac{9 \times 10^6 \text{ counts}}{45{,}000 \text{ counts}/\mu l}$ for 9 ml. = 200 µl
180 µl H.S. DNA

For Blot of NT. 2, NT.3

$\frac{6 \times 10^6}{45{,}000}$ for 7 ml = 133 µl
140 µl H.S. DNA

Total
333 µl F1 probe
320 µl H.S. DNA

[redacted]

Second Gel to Determine Conc. of DMD cDNA's

- Dilutions made of 5 clones (1/10) using 10λ
- Run on same gel (12 aliquots)

-2	-1	3	4	5	6	7
new λ-HindIII	47.4	44.1	9.7	30.1	30.2	63.1

	Conc	Conc
9.7	20ng/µl	
30.2	35ng/µl	20
30.1	30ng/µl	20
47.4	8ng/µl	
44.1	23ng/µl	
63.1	15ng/µl	

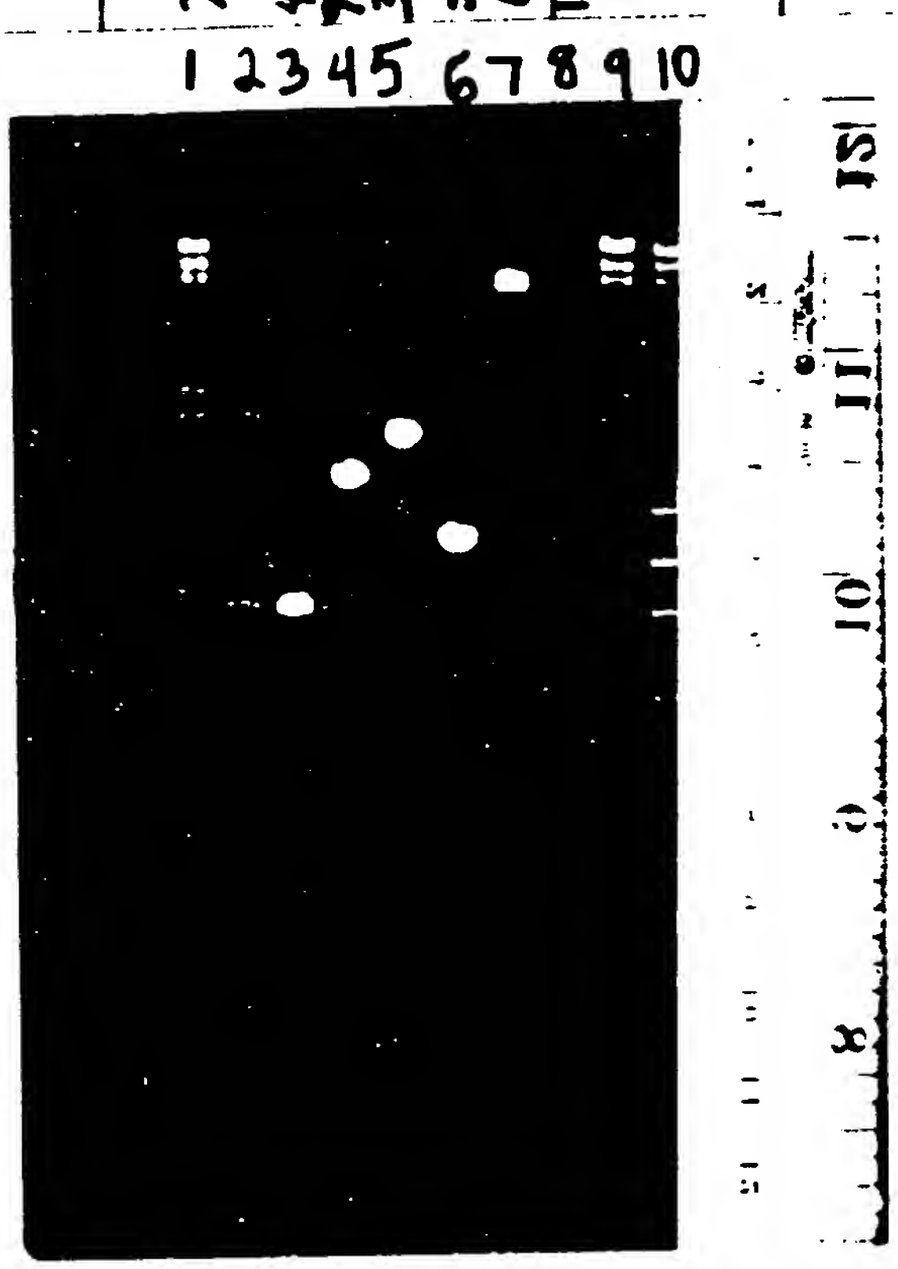
* dilute 30.2 & 30.1 to 20ng/µl = TE

Concentrations range from 20ng (47.4) to 35ng/µl (30.2)

Gel Run to Determine DMD cDNAs' Concentration (inserts)

- Aliquots were diluted from 50λ to 100λ
- 1λ of each was run on a gel with Jeff's λ-Hind III standard.

1	2	3	4	5	6	7	8	9	10
λ-Hind III 5λ	44.1 (before centfg) Hole in well	44.1 (after cfg.)	9.7	30.1	30.2	63.1	12.2 some missed the well	λ-H 5λ	λ-H 6λ



Concentration estimated to be 200-300ng/λ in 5 DMD cDNA's

Isolated Inserts

	clone	2	size	4	RE's	6	7	8	conc.	vo
1										
2	12.2		2.75Kb		Hind/Sal				4ng/λ	10
3										
4	NT.2		3.35Kb		Hind/Sal		} ~ 4 ~ <		30ng/λ	10
5										
6	NT.3		3.2Kb		Eco/Sal		}		18ng/λ	10
7										
8	47.4		0.6Kb		Eco/BglII 3'		} dilute to 20λ ~5			
9							~ ½d agarose (~2			
10	63.1		1.0Kb		Hind				1:10 d.i.c. 2λ	
11										
12										
13	NT 16		4.3 Kb		HindIII		} dilute ~ 20		8ng/λ	10
14										
15	λ20		~~1.9~~ 1.25Kb		HindIII-Sal		}		8ng/λ	10
16										
17										
18	PT218R		2.9		~~HindIII~~	Sal I			120ng/λ	20
19										
20	PT219R		"		~~Sal II~~	HindIII			200ng/λ	20
21										
22	reprecipitated (Phenol extracted)									
23	47.4		0.6Kb		Eco/BglII	3'			20ng/λ	30
24										
25	63.1		1.0Kb		Hind				40ng/λ	30
26										
27										
28										
29										
30										
31										

Add 28 λ
60 λ
TE

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Preparation of Vectors for Subcloning

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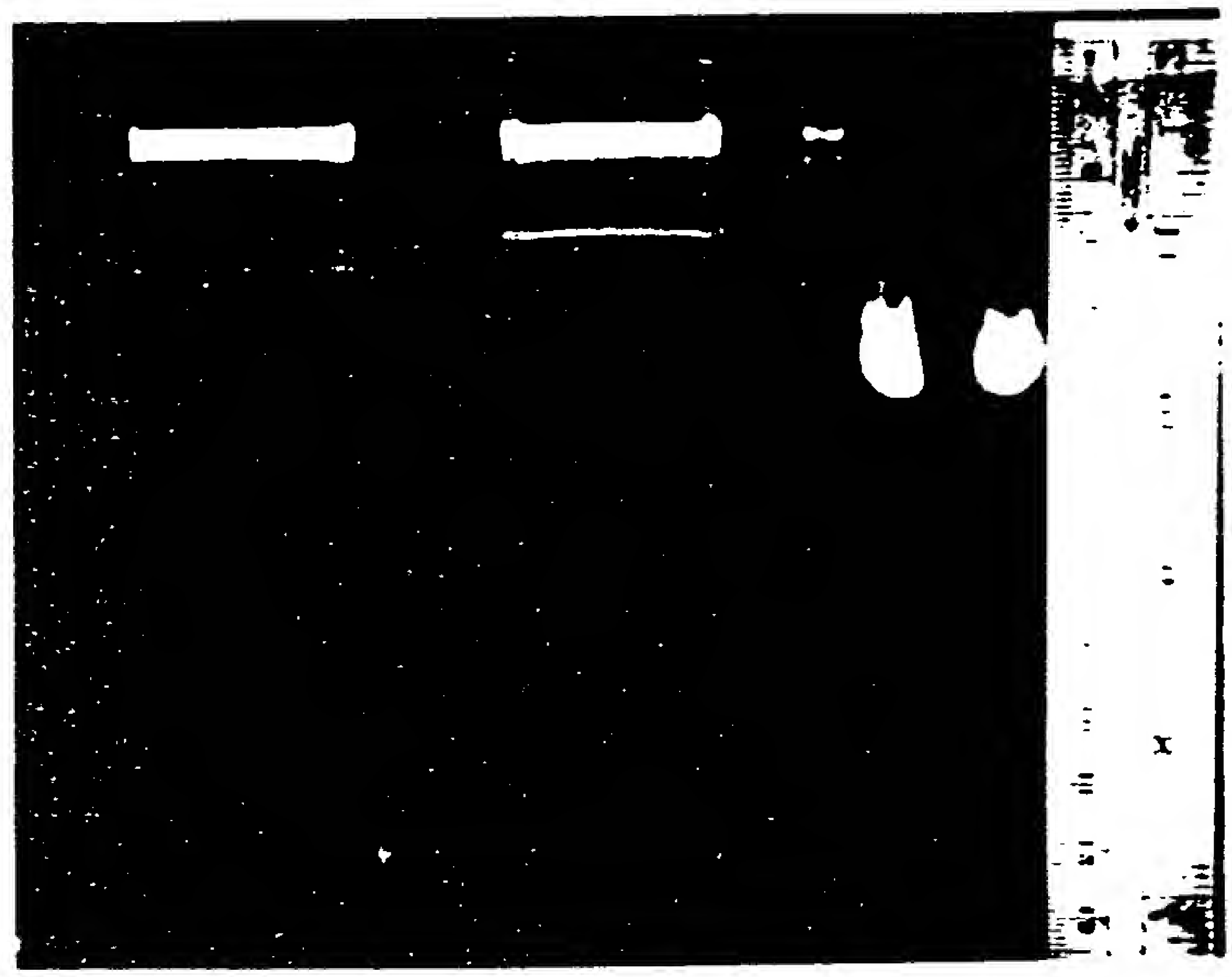
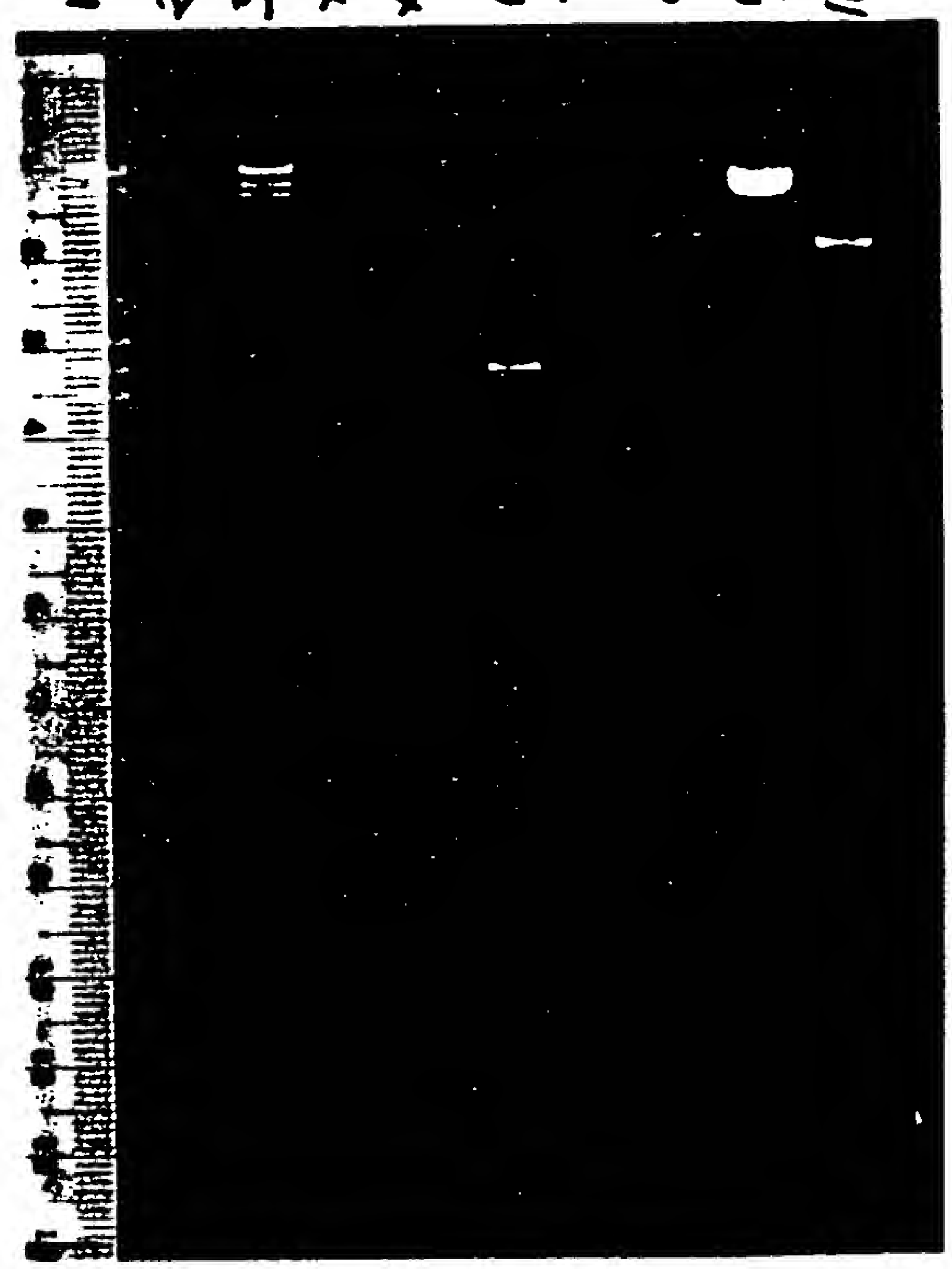
DNA	(µl)	10xBuff	(µl)	Enzyme	(µl)	Spermidine	RNAse A	H2O	Tot
pTZ19R	5	2	5	HindIII	5	2	2	31	50
pTZ18R	5	10	5	SalI	5	2	2	31	50



DNA Quantitation

1 My Standard
2 NT.3i 1.0λ of 10λ
3 Jeff's Standard
4 X
5 X
6 63.1i diluted to 20λ, ½λ run (1.0kb HB)
7 47.4i " (.6kb EBg)
8 NT.2i 1.0λ of 10λ
9 Jeff pRESAC5 insert
10 Jeff pTZ EcoRI ~100ng/λ?

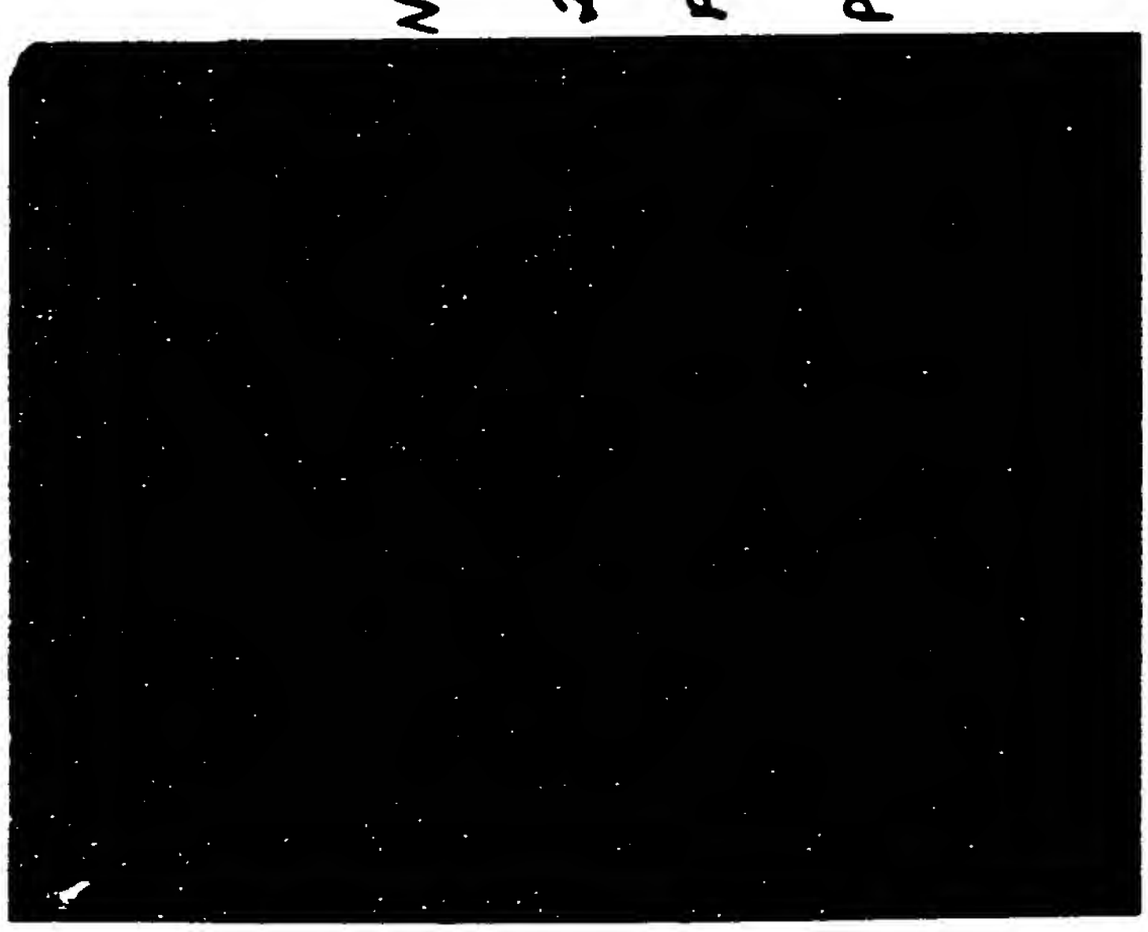
1 2 3 x x 6 7 8 9 10



Gel to Quantitate NT.1, 20, PTZ18R, 19R

	1 Lane	2	3	4	5	6	7	8	9
1	1		My standard						
2	3		NT.1b insert						
3	5		20 insert						
4	7		PTZ18R ~~HindIII~~			SalI			
5	9		PTZ19R ~~SalI~~			HindIII			
6	11		Jeff's standard						
7									
8									
9	~150								
10	X250								
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

NT.1b 20 PTZ18R PTZ19R



AMRAD EFFICIENCY LINE • 22-206

Klenow Fill-in RXN c NT.2,3,20,12.2* [redacted]87

	NT.2,3 & 20	
	(μl)	(μl)
DNA	5	9
dNTPs	1	1
10x Klenow Buffer	1.5	1.5
Klenow	1	1
H_2O	7.5	2.5
	15λ	15λ

1hr. 37°C
½ vol NH_4Ac
2 vol 95% EtOH

*viscous when pipetted
*Will directly ligate NT.1b

9/11/87

Jeff decided to phenol extract, EtOH precip.
Will redo Klenow fill-in

Dissolved in 11 μl H_2O

H_2O+DNA	11
dNTPs	1
10x Buff	1.5
Klenow	1.5
	15μl

37°C 1hr.
EtOH O/N

TCA Precipitation of 47.4, XDSA labelling

After oligolabelling add 50λ .2× SET

Mix

~~Vort~~ Remove 12 from each rxn. - Add to 6ℓ HS DNA

Add ~ ½ mls 10% TCA

Vortex

Ice 5'

Filter thru GF/C filter

Rinse 3-4 times c 10% TCA

Rinse c 3 mls 95% EtOH

Dry (air)

Count in thin scint. vials c filter in toluene

9/10 Unsuccessful — due to use of α-P^{32} CTP instead of ATP

9/11 " — CTP instead of dCTP

Repeat O/N

Ligation of NT.1b, 2, 3, 20, 12 into pTZ [redacted] 87

Centrifuged DNA from Blunt-end rxn.
Added 100µl 70% EtOH
Resuspended in 6½λ H_2O

For Blunt-end Ligations

DNA(+H_2O)	6.5λ
10x Ligase Buff	1λ → in 'digobox'
10mM ATP	1λ
T4 Ligase	1λ → ē restriction enzymes
vector	½λ → HincII cut pTZ, Box 7, 6H
	10λ

O/N R.T.

For HindIII Ligation NT.1b insert

DNA+H_2O	5λ	
10x Ligase Buff	1λ	Mix gently (like ē Klenow)
10mM ATP	1λ	
T4 Ligase	½λ	
pTZ19R-HindIII	1λ	(50ng/λ)
H_2O	1½λ	
	10λ	

O/N 4° - cold room

Transformation of DH5α F' [redacted]/87

IPTG 10λ
XGAL 25λ

Removed tube of (.5ml) DH5αF's - thawed on ice
Transfered 20μl to 5 tubes * pre-cooled
Added 2μl of a 1/10 dilution of Ligations
* Gently pipetted up and down

should have gently flicked to mix

Aliquotted 100μl to microfuge tubes → -70°C (quick fr... in fros...
Let sit on ice 30 min.
Heat shocked 40 sec 42°C
On ice
Added 80μl S.O.C.
Incubated 1 hr. 225 r.p.m.
Plated out c̄ 10λ IPTG, 25λ XGAL, all samples on Amp plates

All had positives except NT.3

Concentration Determination of Phenol extracted

	1	2	3 47.4 0.6Kb E/Bgl 63.1, 1.0Kb H3	6	7	8	9
1							
2							
3							
4							
5							
6					47.4	0.6Kb	Eco/Bgl 20ng
7							
8					63.1	1.0Kb	H3 40ng
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							
19							
20							
21							
22							
23							
24							
25							
26							
27							
28							
29							
30							
31							

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Hybridization of Hu Gen. Lib. vs. 87

1	2	3 47.4-0.6 Kb E/Bg Fragment	8	9

Pre-hyb. - 80 mls → No Dextran Sulfate
H.S. DNA

Hyb. - 200 µl probe + 1.6 mls H.S. DNA
(~2 x 10⁶ counts)

TCA precip. - Let go O/N
Repeated TCA precip.

* → Results Negative for screening of filters @ 47.4-0.6 Kb E/Bg
(lifted @ 7/16/87)

9/14 [illegible] A=351954.5(0.3%) B=351915.0(0.3%) C=000000.0(>20%) S=1

9/15 [illegible]2.00 A=130476.5(0.5%) B=130460.5(0.5%) C=000000.0(>20%) S=1.000

9/19 351954 x 50 x 1 = 176 x 10⁶ counts

9/15 130460 x 100 = 13 x 10⁶ counts
(Repeated rxn ⊂ phenol extracted 47.4 .6Kb E/Bg + 63.1 1.0Kb HB

* Pooled all 47.4 labellings (2) 13 x 10⁶
18.5 x 10⁶
31.5 x 10⁶ counts

Next Page

47.4 0.160 E/BGI

[redacted]

185 06 X 100 X 1 = 18.5 x10⁶ counts

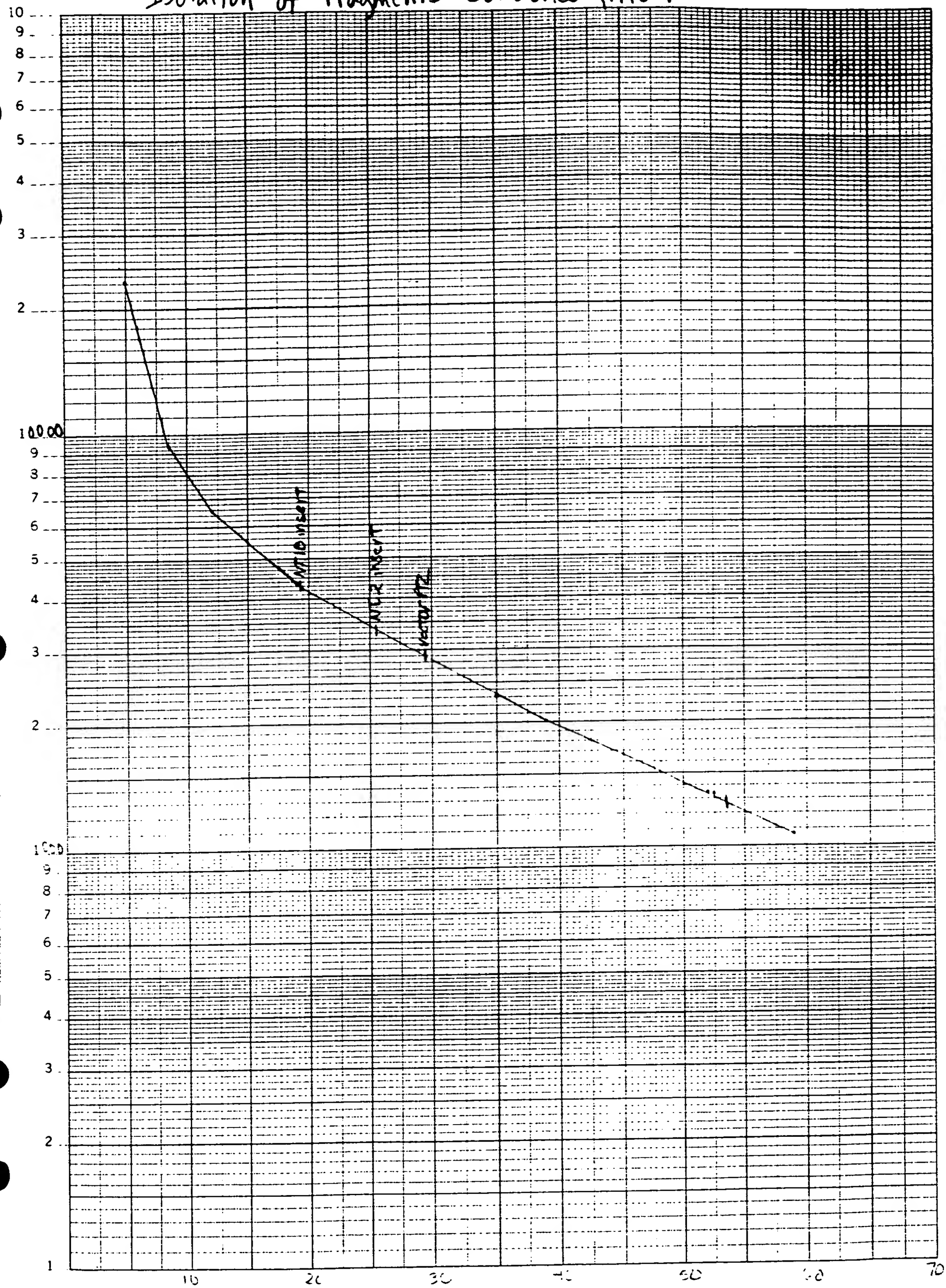
63.1 1.046 Hz

[redacted]

56068 x100 x1 = 5.6 x10⁶ counts

Did not label well
After Lisa labelled it- no problem

Isolation of Fragments Subcloned into PTZ



46 5810

K·E SEMI-LOGARITHMIC, 11 CYCLES - 140 DIVISIONS
KEUFFEL & ESSER CO. MADE IN U.S.A.

Mini-Prep (Gen. clone inserts, DMD inserts in PTZ)

Make soln 2 Fresh → 3.8ml → 4.0ml

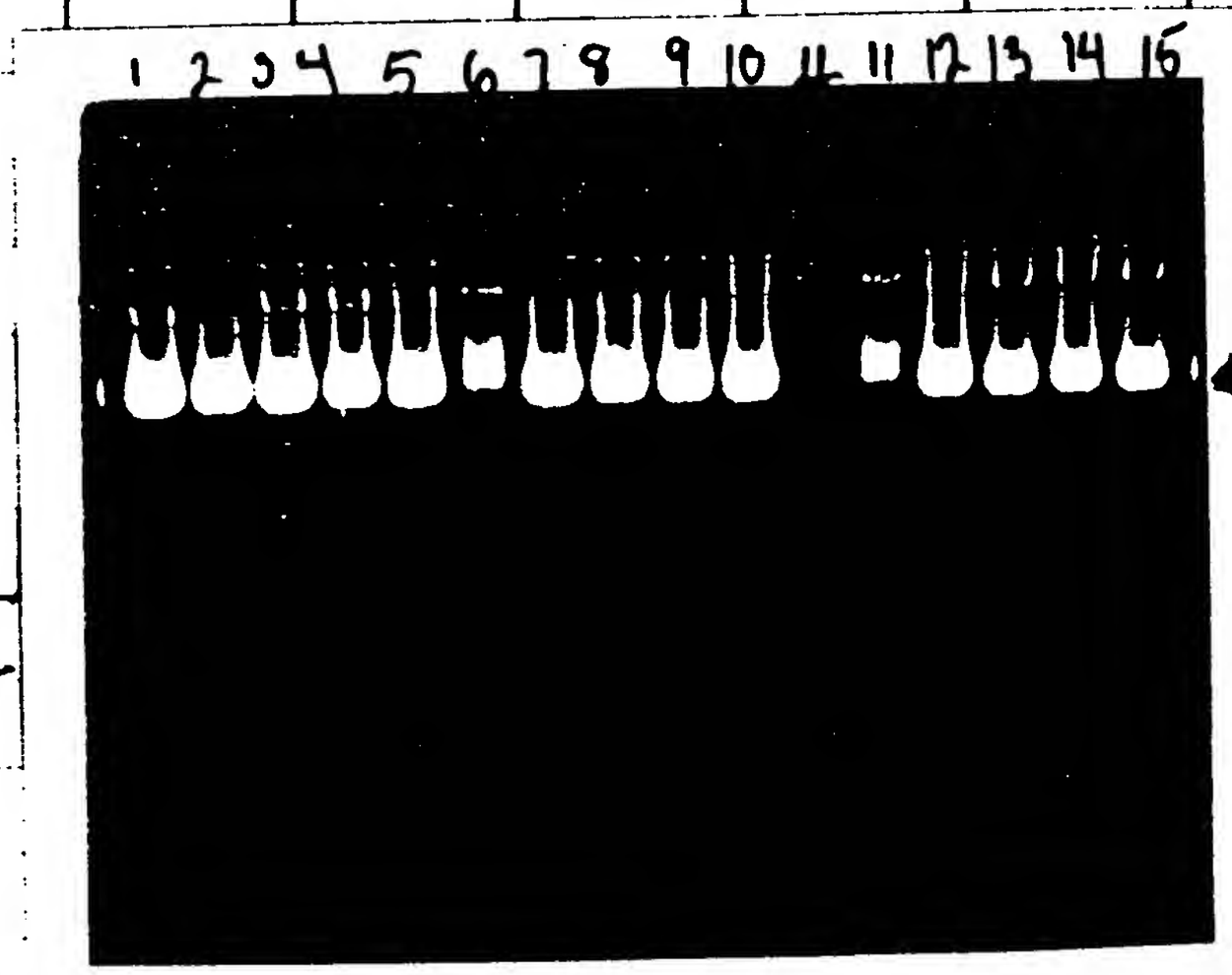
80µl 10N
160µl 25% SDS
3760µl H_2O

60µl 10N NaOH
120µl 25% SDS
2820 H_2O

Soln. 1 need 2 ml
" 3 " 3 ml

1	20	I
2	20	II
3	20	III
4	20	IV
5	20	V
6	20	VI
7	12.2	I
8	12.2	II
9	12.2	III
10	12.2	IV
11	12.2	V
12	12.2	VI
13	NT.2	I
14	NT.1b	I
15	NT.1b	VI

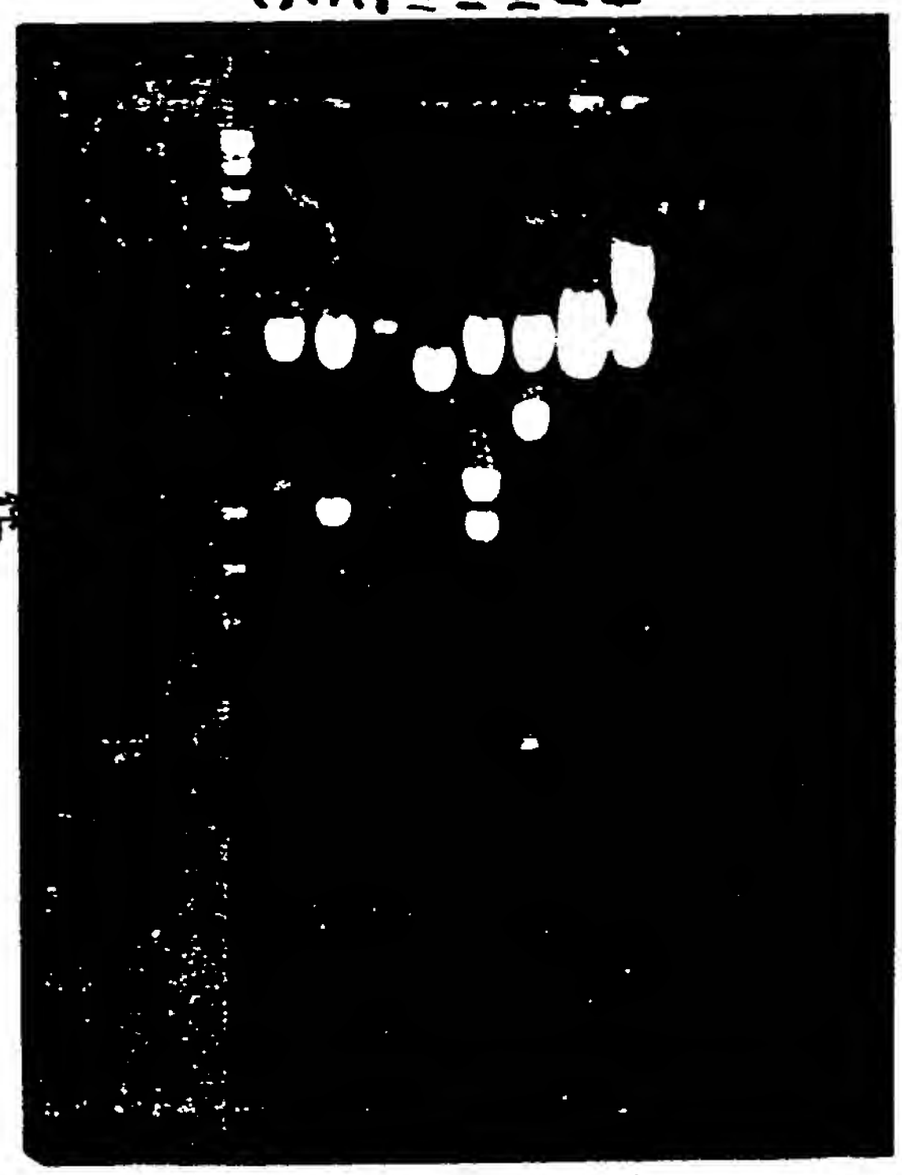
Some soln spilled (NH_4Ac + DNA)



Added NH_4Ac instead of NaAc (soln 3)

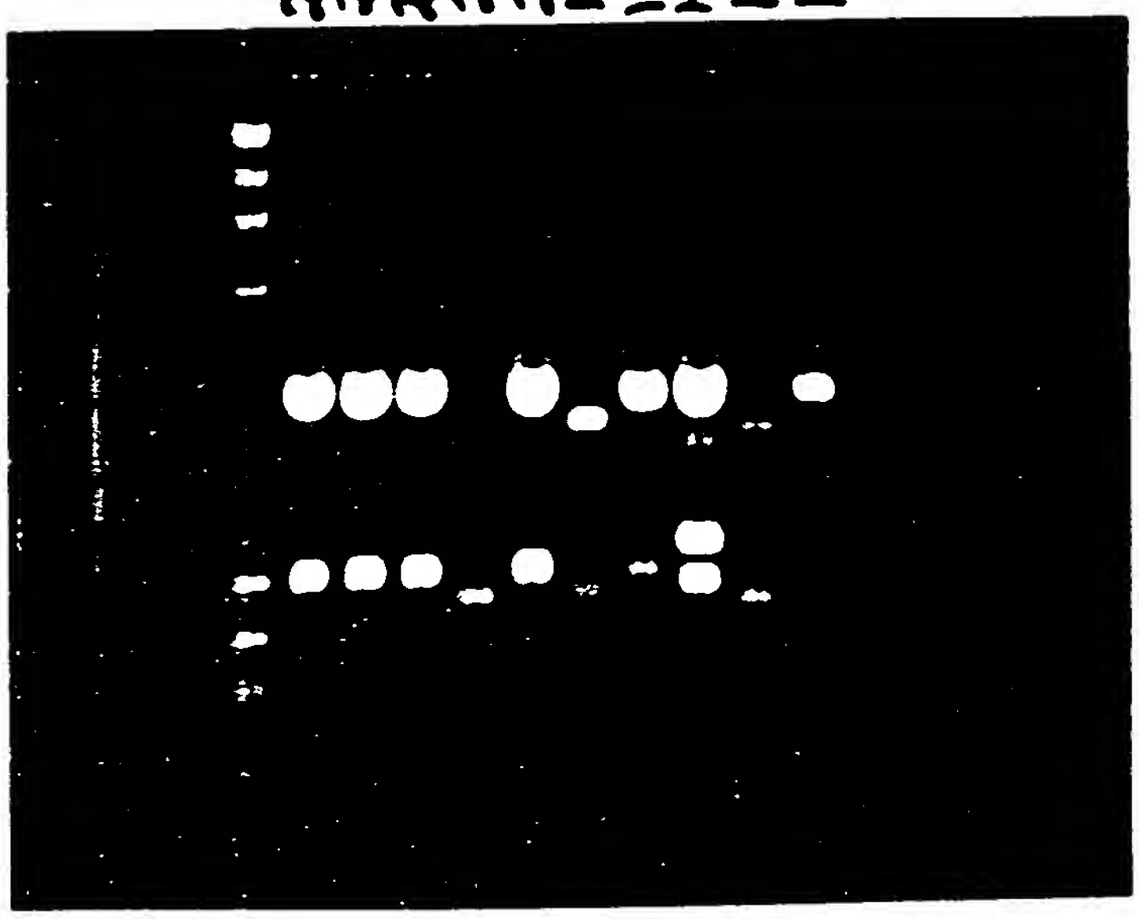
Mini-Prep Gel ε NT.1b, .2, 20, 12.2's

Lane		DNA	µ(µl)	10xEcoRI Buffer µ(µl)	Enzyme µ(µl)		Spermidine	RNAse A	TE	To
1	1	STANDARD		15						
2 √ (1)	2	20	10	2	2	HIII/SST	1.0	1.0	1	2	3	2
(2)	3	20		2	2	"	"		1	2		
(3)	4	20		2	2	"	"		1	2		
(4)	5	20		2	2	"	"		1	2		
3 √ (5)	6	20	10	2	2	"	"		1	2	3	20
4 √ (6)	7	20	10	2	2	"	"		1	2	3	20
(7)	8	12.2		2	2	"	"		1	2		
(8)	9	12.2		2	2	"	"		1	2		
5 √ (9)	10	12.2	10	2	2	"	"		1	2	3	20
6 √ (10)	11	12.2	10	2	2	"	"		1	2	3	20
(11)	12	12.2	1	2	2	"	"		1	2		
7 √ (12)	13	12.2	10	2	2	"	"		1	2	3	20
8 √ (13)	14	NT.2	10 lg. scale prep	2	2	"	"		1	2	3	20
(14)	15	NT.1b		2	2	Hind	2		1	2		
9 √ (15)	16	NT.1b	10 lg. scale prep	2	2	Hind	2		1	2	3	20
	17											
	18	Digest 2 hrs.										
	19											
	20	20	1.25Kb									
	21	12.2	2.75Kb									
	22	NT.2	3.35Kb									
	23	NT.1b	4.3Kb									
	24											
	25											
	26											
	27											
	28											
	29											
	30											
	31											



Second Mini-prep of Ligations → PIZ

	1	2	3	4	5	6	7	8	9
1	20	VII							
2	20	VIII	→ missing from freezer						
3	20	IX							
4	20	X							
5	20	XI							
6	20	XII	Lg. scale prep						
7	12.2	VII							
8	12.2	VIII							
9	12.2	IX							
10	12.2	XI							
11	12.2	XII							
12									
13									
14	12.2	X did not grow							
15									



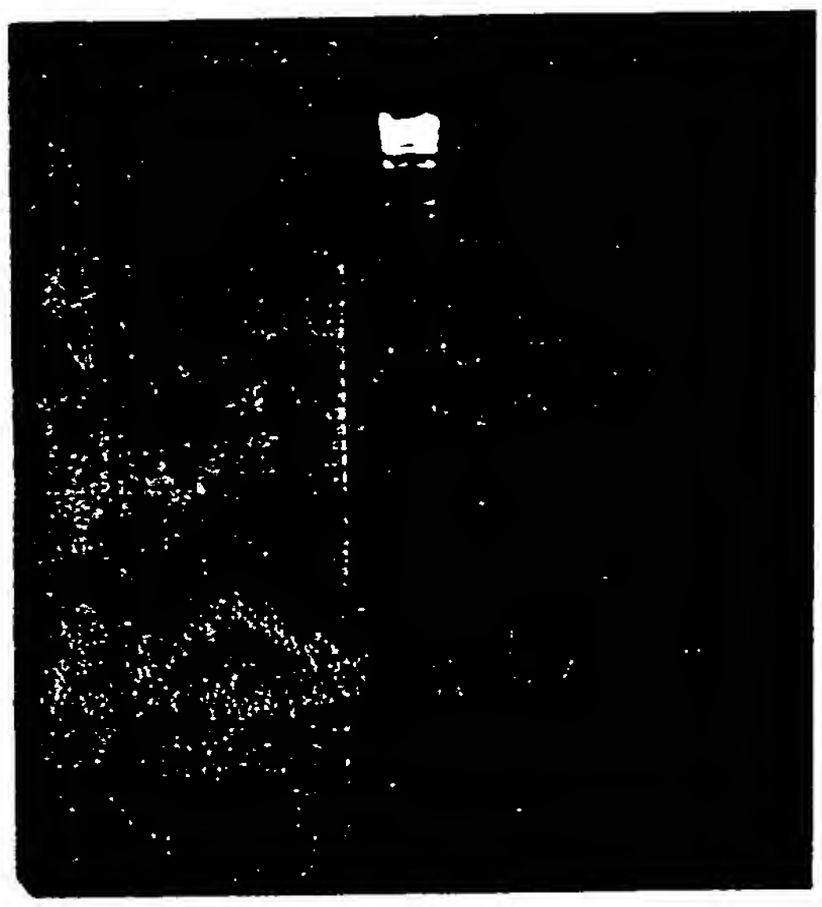
	DNA	(µl)	10x Buff	µl	Enzyme	µl	Spermidine	RNase A	TE	Tot
0	λ-Dx	15								
1	5	1-20 VII	React 2	2	H3/SST	1+1	1	2	8	20
2	5	3-20 IX	2	2	H3/SST	1+1	1	2	8	20
3	5	4-20 X	2	2	H3/SST	1+1	1	2	8	20
4	5	5-20 XI	2	2	H3/SST	1+1	1	2	8	20
5	5	6-20 XII	2	2	H3/SST	1+1	1	2	8	20
6	5	7-12.2 VII	2	2	H3/SST	1+1	1	2	8	20
7	5	8-12.2 VIII	2	2	H3/SST	1+1	1	2	8	20
8	5	9-12.2 IX	2	2	H3/SST	1+1	1	2	8	20
9	5	10-12.2 XI	2	2	H3/SST	1+1	1	2	8	20
10	5	11-12.2 XII	2	2	H3/SST	1+1	1	2	8	20
11										
12	Digest 2 hrs.									
13	1% gel									
14	Blotting									

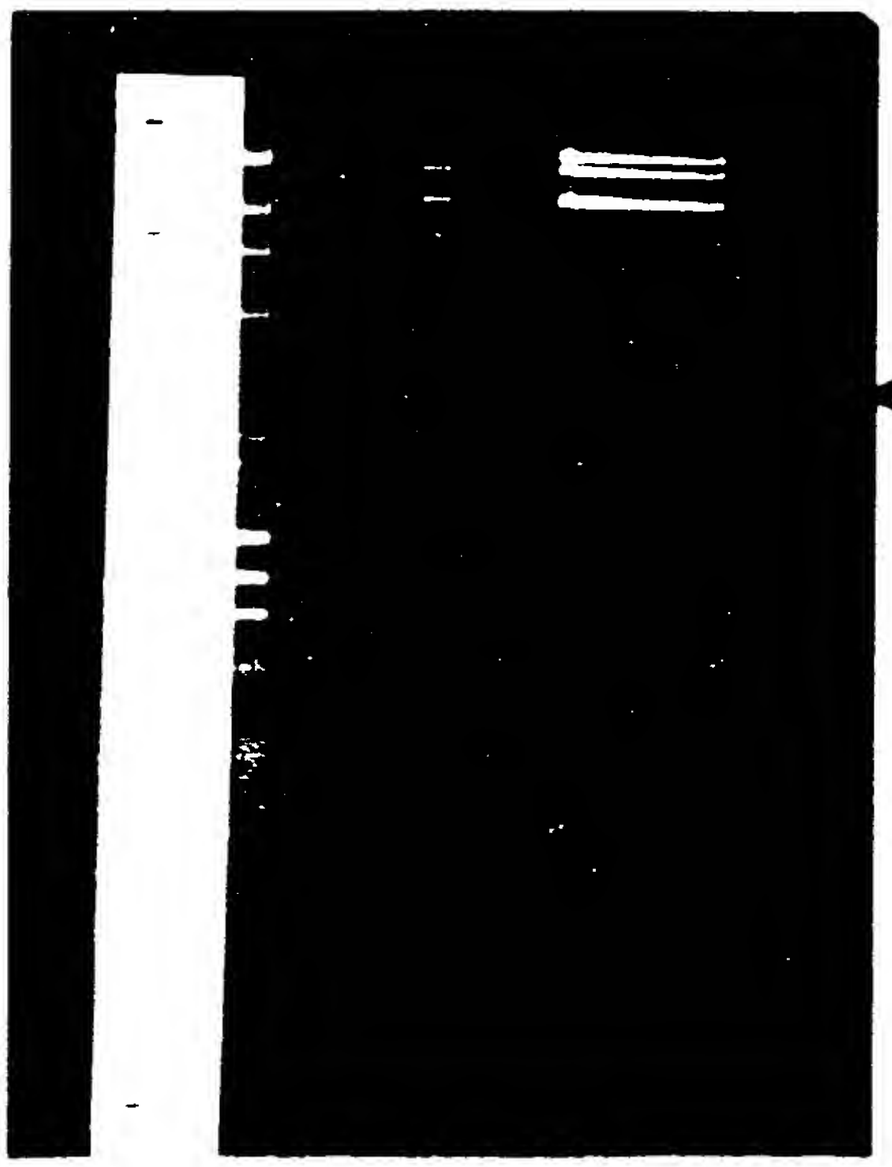
Prep Gel to Isolate 3Kb (Asp718) Frag. of 12.1

[redacted] 87

	1 DNA	2 (µl)	3 10x Buff	4 (µl)	5 Enzyme	6 (µl)	7 Spermidine	8 RNAseA	9 TE	
1	λ-ØX	20								
2	12.1	10	2	5	Asp 718	5	2	3	25	50
3	12.1	~250	2	40	Asp 718	40	20	20	30	400

Cut out 3Kb Asp 718 fragment
Soaked in H_2O 30' - shaking ~ every 5'
Set up electrolution





12.1
3Kb Asp

Concentration of 12.1 3Kb Asp frag
20-25 ng/λ.

Jack Rainier

Klenow Fill in Rxn. For 12.1 3Kb Asp (25ng/λ) [redacted]

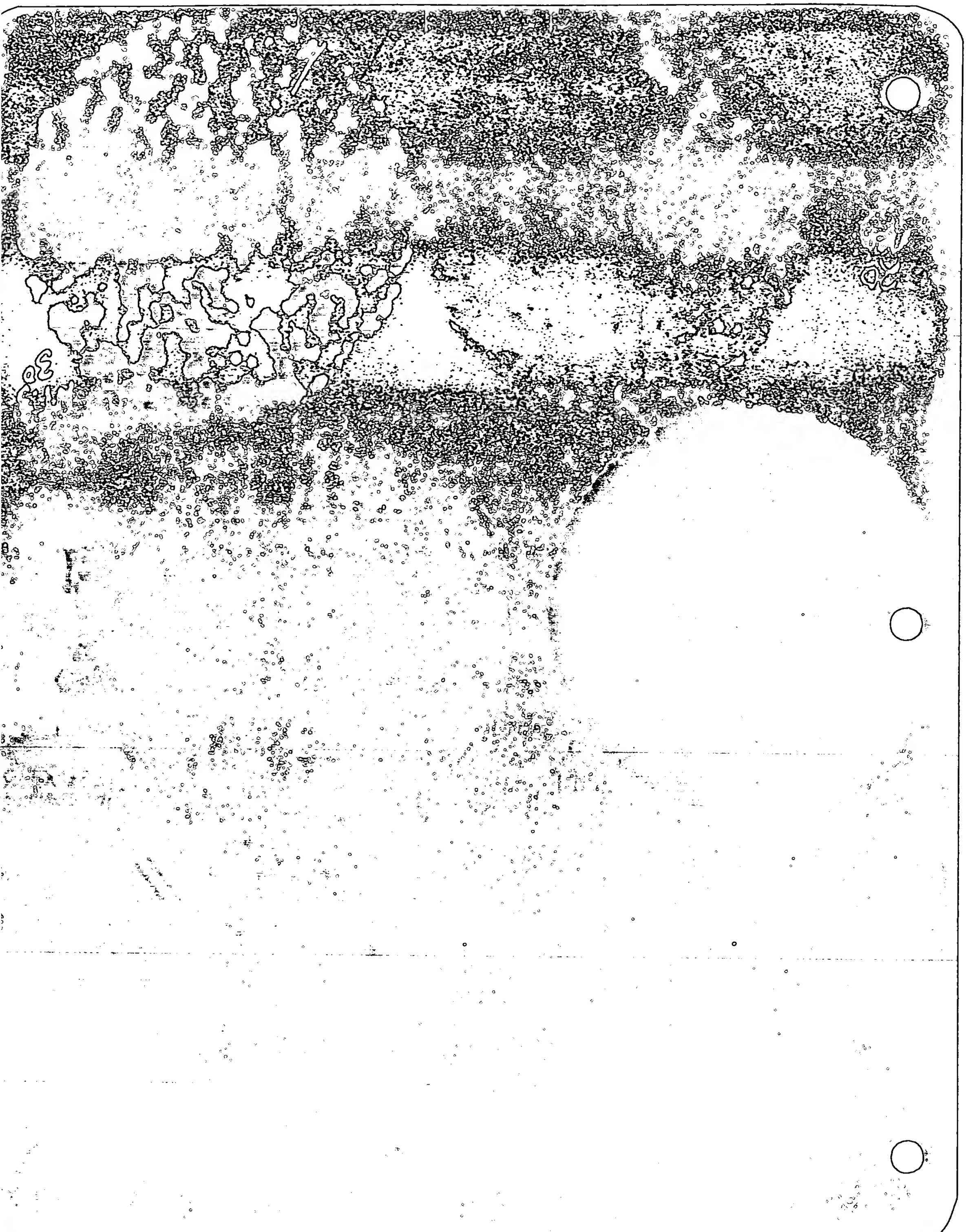
H_2O+DNA	5 μl	
dNTP's	1 μl	
10x Buff.	1.5 μl	
Klenow	1 μl	
H_2O	6.5λ	
	15λ	

½ vol Amp
2-2½ vol. EtOH - mix
-70°C 10 min.
Wash ĉ 50λ 70% EtOH
Resuspend in vol. to use for Ligation

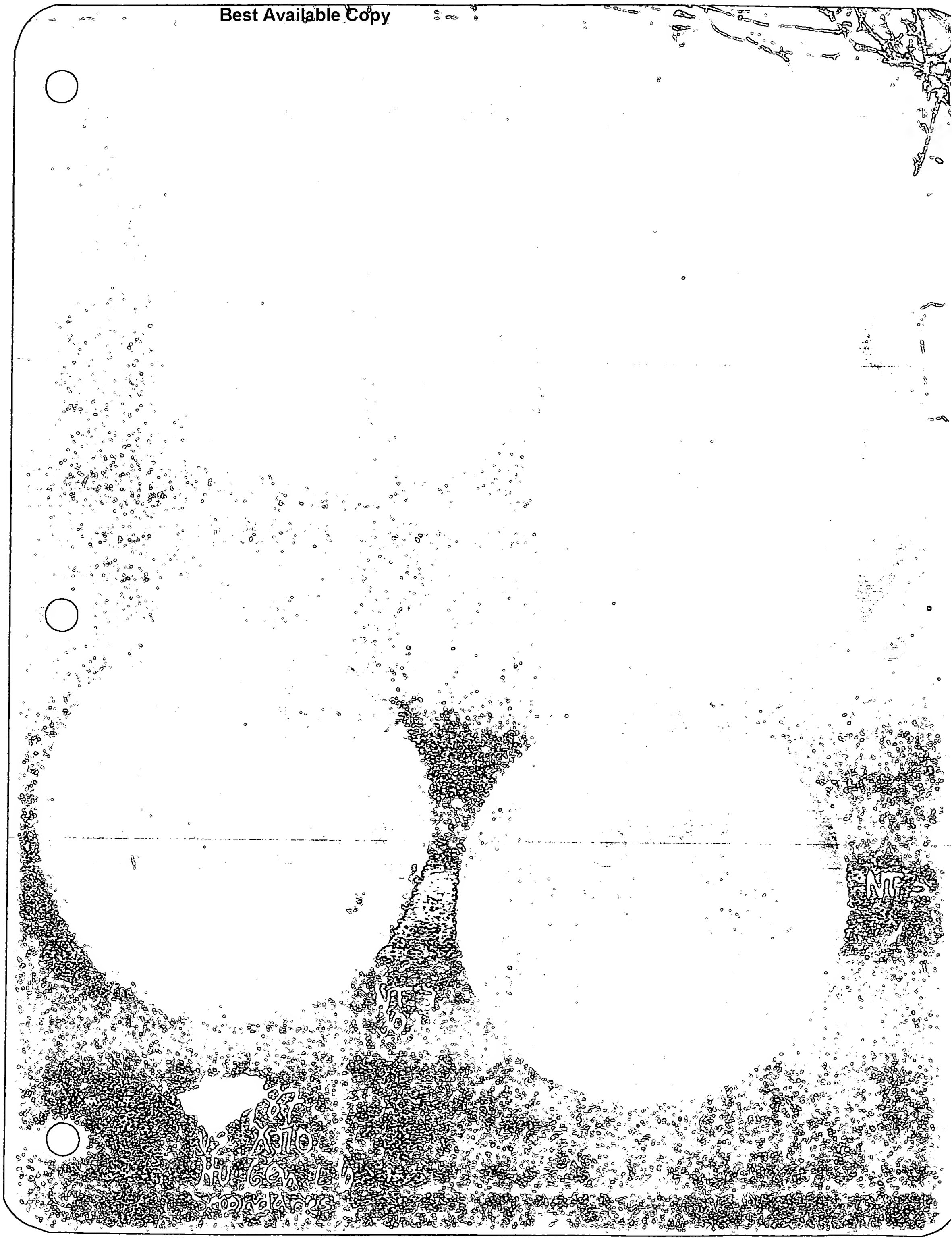
*Could have done 10λ rxn

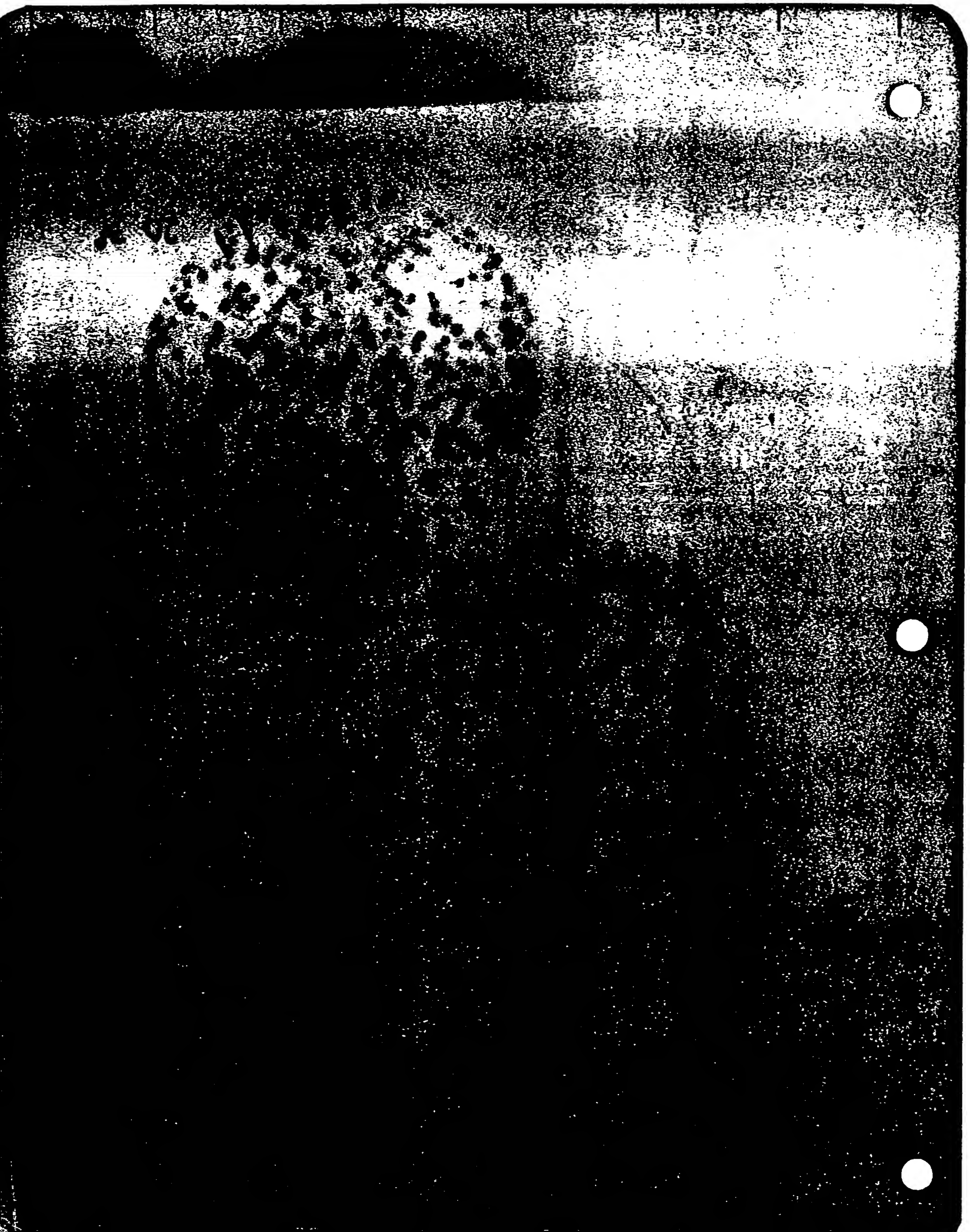
Ligation of 12.1 3Kb A into PTC18R

H_2O+DNA	6.5 μl
10mM ATP	1 μl
Vector	½ μl
T_4 10x Buff	1 μl
Ligase	1 μl




A.2
1/100 SA 19.2
AMD 19.2
1ST pick





Digestion of 47-4 c HincII [redacted]

2.4 mg/ml - cut 6 λ c EcoRI, HincII, Toyoba kit-ref

DNA	6 λ		
10× Li	10 λ		
SP	4 λ		
RNase	2 λ		
EcoRI	4 λ	10 u/λ	BRL
HincII	6 λ	40/λ	NEB
H_2O	68 λ		
	100 λ		5:40

20 λ each
10 λ to 15A

.05 o.c 47-4 EcoHincII

[redacted]

~3.46 10/16

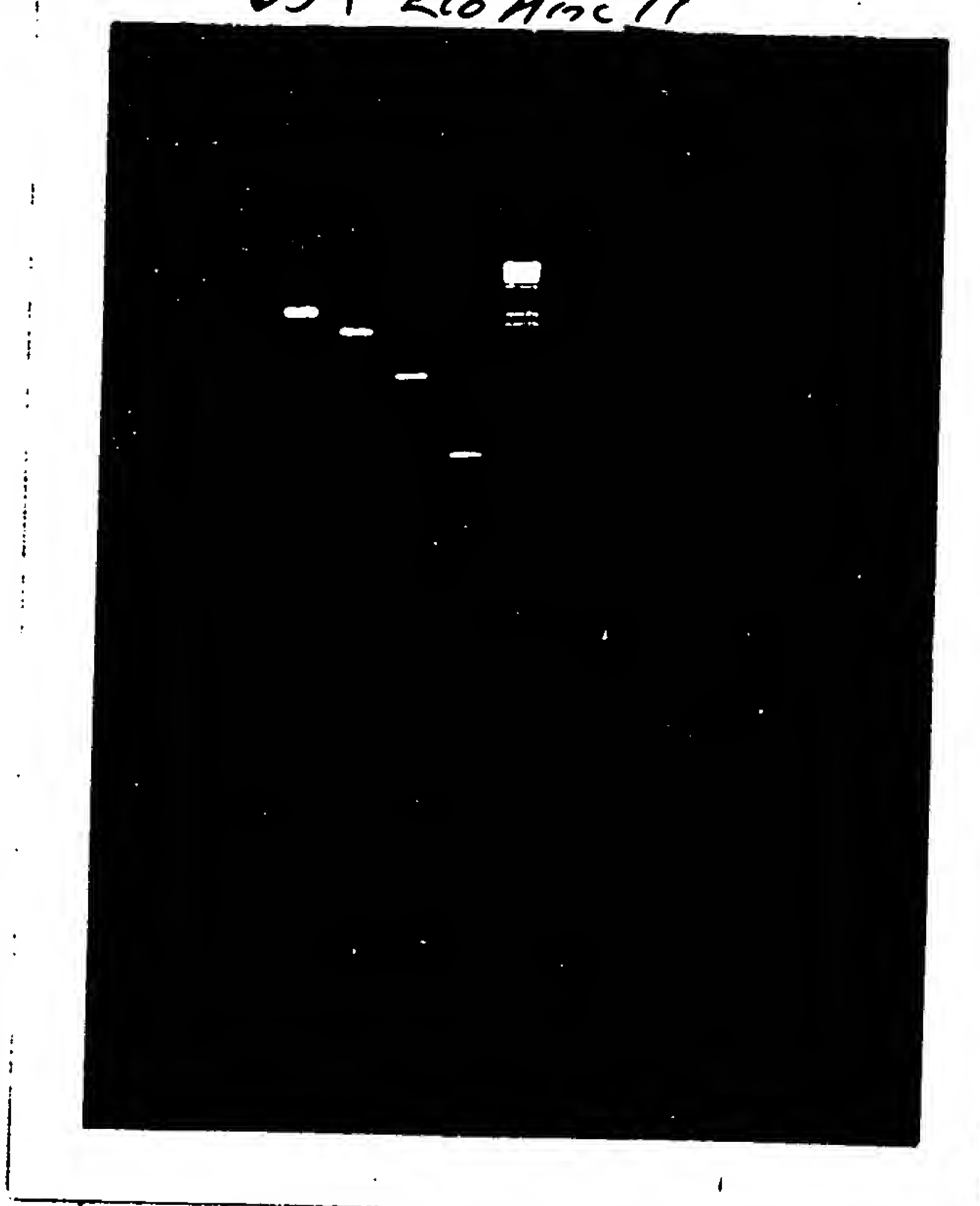
Digestion 63-1 c̄ Eco·HincII [redacted]

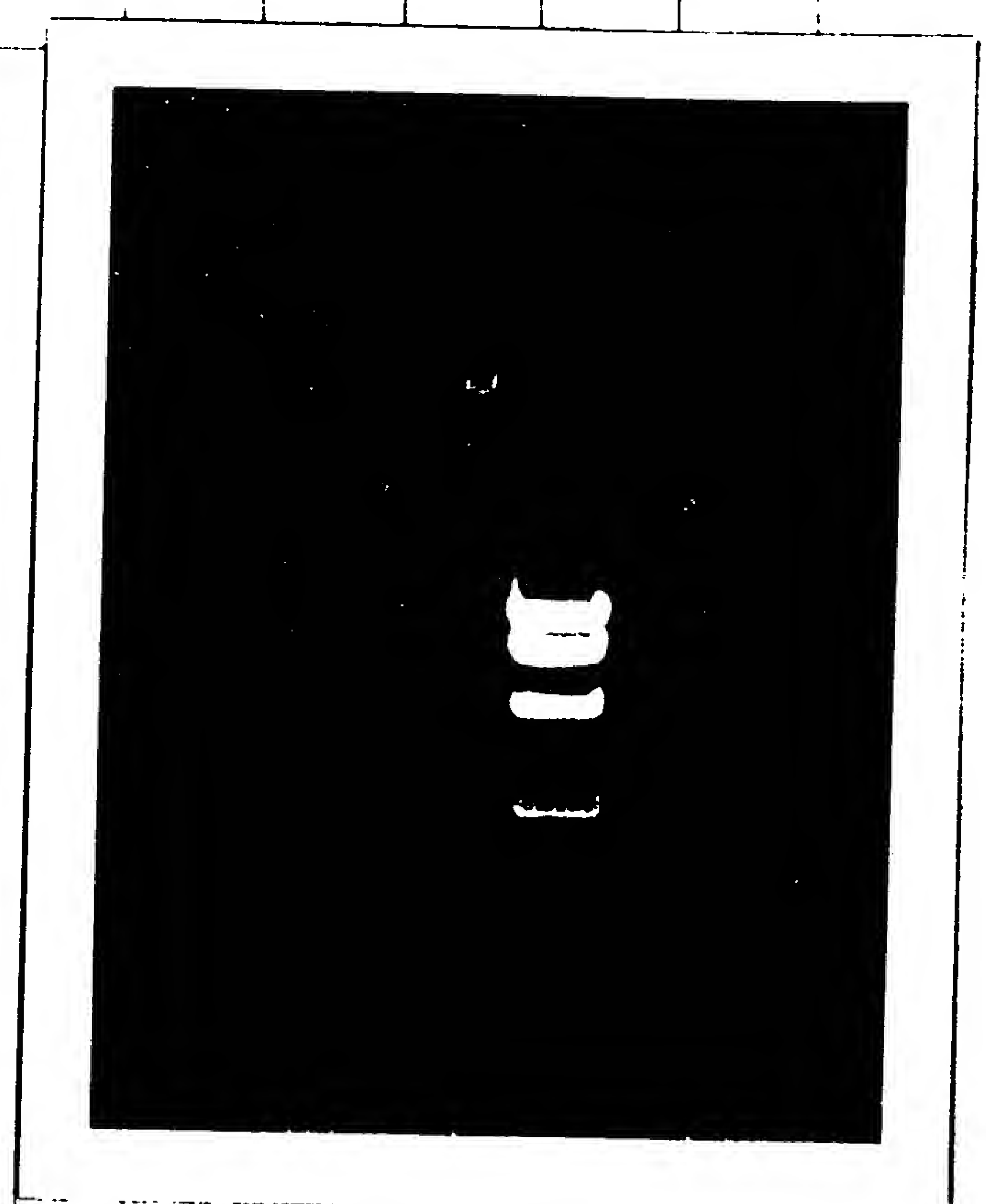
~15 µg Toyoba hi salt, 100 µl rxn
electroelute 4 bands (bottom 4, vector as top one)

~25% yield

2.5 kb	100 ng/µl	20 µl of each
1.9 kb	60 ng/µl	10 µl to LSA
1.05 kb	40 ng/µl	
~.6 kb	20 ng/µl	

63-1 EcoHincII





Large Scale Plasmid Prep of Fragments Containing Exons Pulled out c XD-1, XJ10

[redacted] 187

for 100mls soln. 2

0.2N NaOH	2ml	10N NaOH
1% SDS	4ml	25% SDS
	94ml	H_2O

100ml soln 1

50mM	Glucose	5ml 1M
10 mM	EDTA (pH 8.0)	2ml .5M
25 mM	Tris-HCl (pH 8.0)	2.5ml 1M
H_2O		90.5

LYSOZYME

60mg/4ml

Problem c protein / RNA precipitating after loading into tubes for Vti 50
Spun down debris, Extracted c IsoAmyl 4x = vol.
Dialyzed o/N
*Added 80λ 5M NaCl (For better sep. of layers) → Concentrated c Butanol (filled to top of tube) to 4ml.
Extracted 3 samples c = vol. phenol (NT.1b, NT.2, 12.1)
" all samples c 2x vol. chloroform (*Removed Butanol)
Precipitated c 2x vol. EtOH
Washed
Resus. in 500λ

3/200 dil	A_{260}	A_{280}	$\frac{A_{260}}{A_{280}}$	Conc.	Yield
NT.1b	1.222	.576	2.12	4.1 mg/ml	2.0 mg
NT.2	.936	.440	2.12	3.12 mg/ml	1.6 mg
NT.3	.156	.053			
20	.584	.273	2.14	1.9 mg/ml	950 µg
12.1	1.205	.580	2.07	4.01 mg/ml	2.0 mg
6/200					
NT.3	.363	.204	1.78	0.61 mg/ml	303 µg

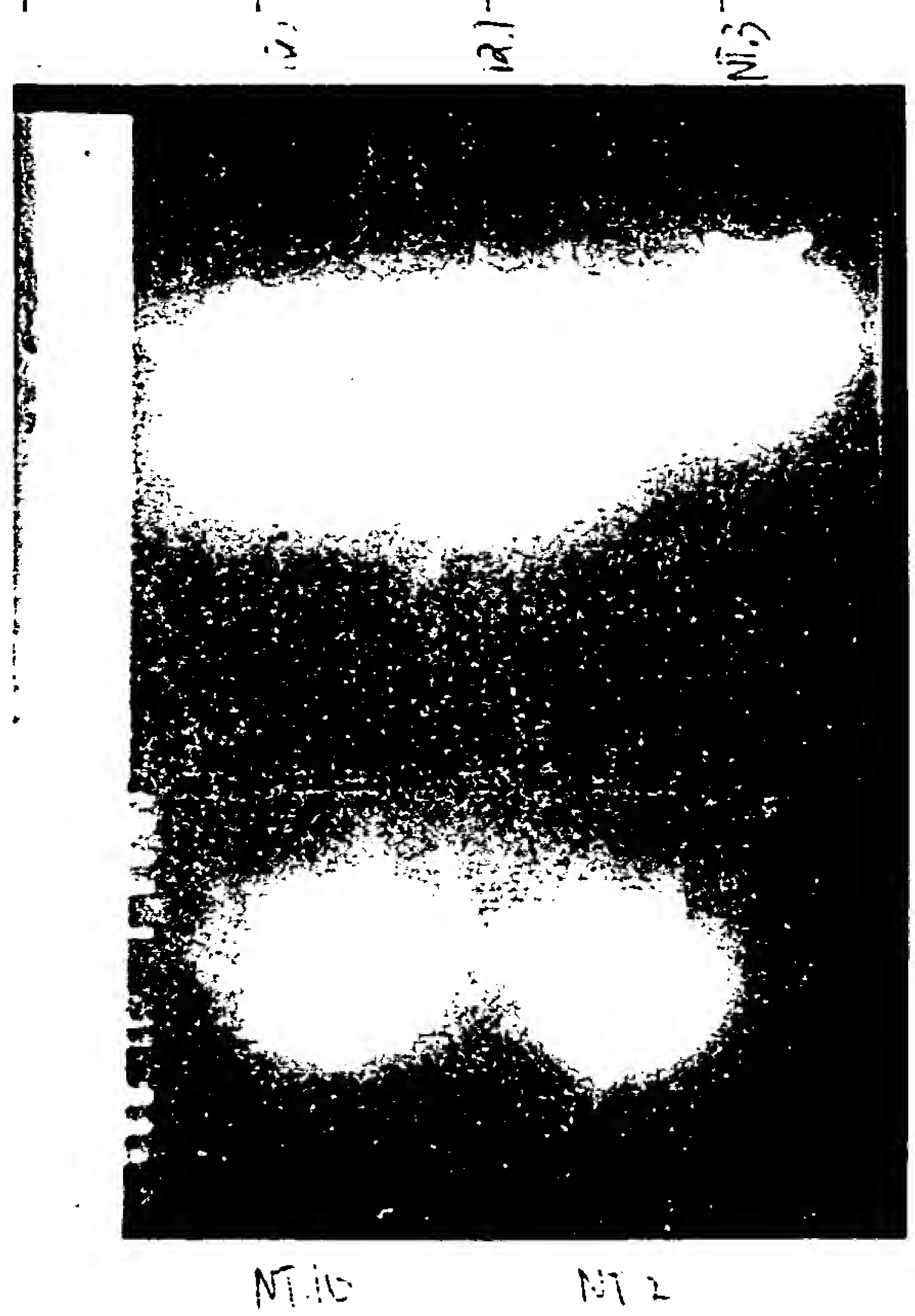
Jorel Kanier

Hu. Gen. Clones (DMD) in PTZ19R [redacted] 87

		Insert	Conc.	For 10µg of insert	Conc of insert		↓	RE sites
NT.1b		4.3Kb	4.1mg/ml	(60%)	2.46mg/ml		4.1µl	HindIII
NT.2		3.35Kb	3.12mg/ml	(53.6%)	1.67mg/ml		6.0µl	H3/Sst
NT.3		3.2Kb	610µg/ml	(52.5%)	320µg/ml		31.3µl	H3/Sst
12.1		3.0Kb	4.0mg/ml	(50.8%)	2.03mg/ml		4.9µl	H3/Sst
20		1.25Kb	1.9µg/ml	(30.12%)	572µg/ml		17.5µl	H3/Sst

cut 20µg insert
- 100λ digestion
- 10X '2' buffer
4λ Spermidine
2λ RNase A
5λ ea. enz, (10λ c̄ ...

	0.2,0.3,20,12	NT.1b	+ 8.2 DNA
Buff	10x4	10	\
Enz H	5x4	10	
S	5x4	—	
Sper.	4x4	4	
RNase	2x4	2	
H_2O	19.4x4	65.8	
	37.4 in each		



	NT.1b	NT.2	NT.3	12.	
H_2O	X	50.6	—	52.8	27.6
DNA	X	12µl	62.6	9.8	35µl

*NT.2 + NT.3 were not resolved
DNA migrated strangely - curling beginning to be visible here

Hybridization of Duble Blot (XJ10, XD-1, 4xY) 11/2/87

Labelling of XJ10 and XD-1 (TCA precipitated)

~~XD-1~~ T=002.00 A=137562.5(0.5%) B=137536.0(0.5%) C=000000.0(>20%)
~~XJ10~~ T=002.00 A=269291.5(0.3%) B=269260.5(0.3%) C=000000.0(>20%)

XD-1 (10% hxn+.25ET)
137526 x100 = 14x10⁶ counts
For 500,000 counts/12 ml used 43.6λ
240λ H.S. DNA

XJ10 (100%, xn+.25ET)
269291 x100 = 27x10⁶ counts
For 500,000 counts/12ml used 23λ

(Both probes thrown in)
Hybed at 50% F
Washed 1x 2xSSC RT
1x 2xSSC 65°C 30'
1x 0.1xSSC 50°C 30'
ON Film O/N RT
& O/N -70°C

4xY (column) Hybed at 50% F
F1: 98882 ÷3 x975 x2 = 64x10⁶ counts → 180λ for 1.2x10⁶/12ml
66,000/λ 240λ H.S. DNA
F2: 42,283 ÷3 x x2 =

T=002.00 A=098882.5(0.5%) B=098872.5(0.5%) C=000000.0(>20%)

T=002.00 A=042283.5(0.7%) B=042275.5(0.7%) C=000000.0(>20%)

Washed 1x RT
1x 0.1xSSC 45,0C 30'

Joel Ranier

Isolation of Hu. Gen. Clone Fragments to be sequenced 14/5/87

	(1) DNA	(2) (µl)	(3) 10xBuff	(4) (µl)	(5) ENZYME	(6) (µl)	Sp.	H_2O	total
1	ΦX	20							
2	NT.1b	10	2	2	DdeI	2	1	5	20
3	4.3kb								
4	20	10	1	2	RsaI	2	1	5	20
5	1.25kb								
6	12.1	8	2	2	HhaI	2	1	3	20
7	1.1kb								
8									
9	Want	NT.1b 0.8Kb DdeI fragment							
10		20 0.6Kb RsaI " .65							
11		12.1(1.1) 0.45Kb HhaI "							
12									
13									
14									
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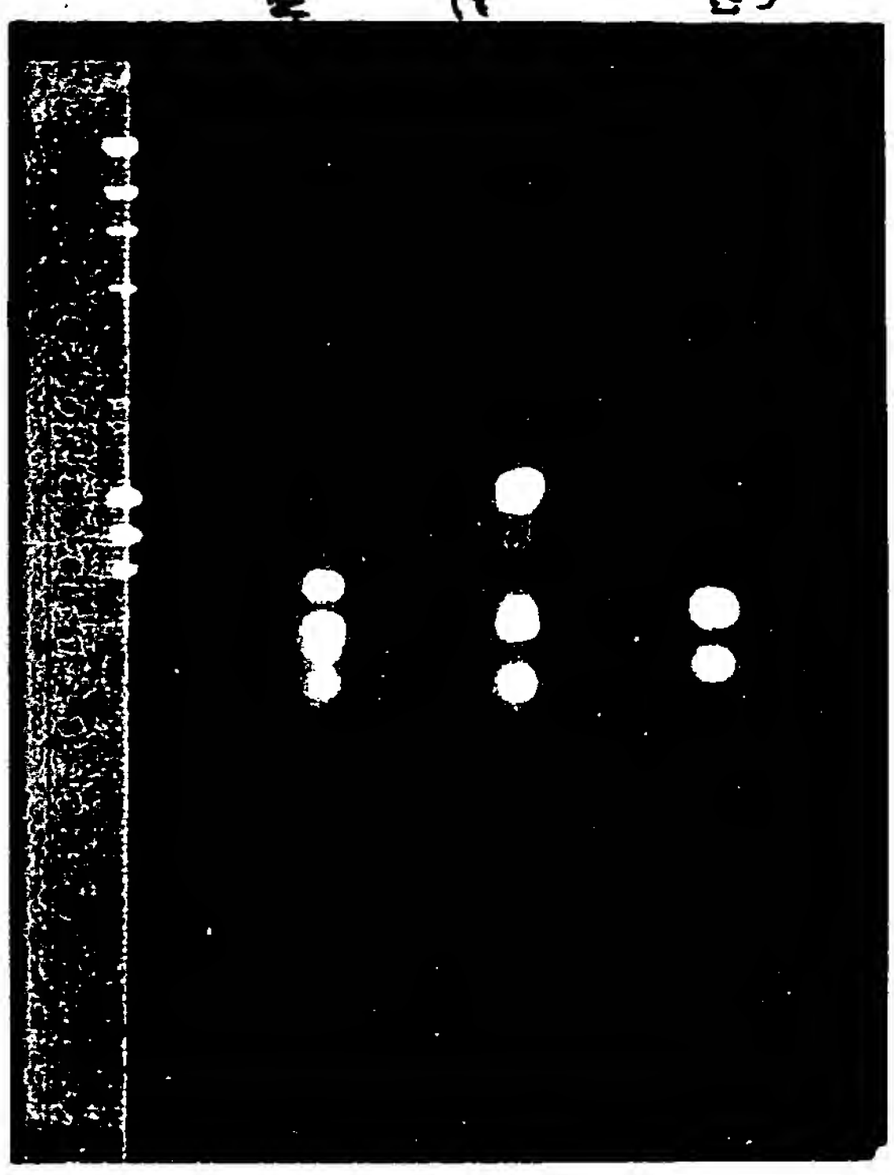
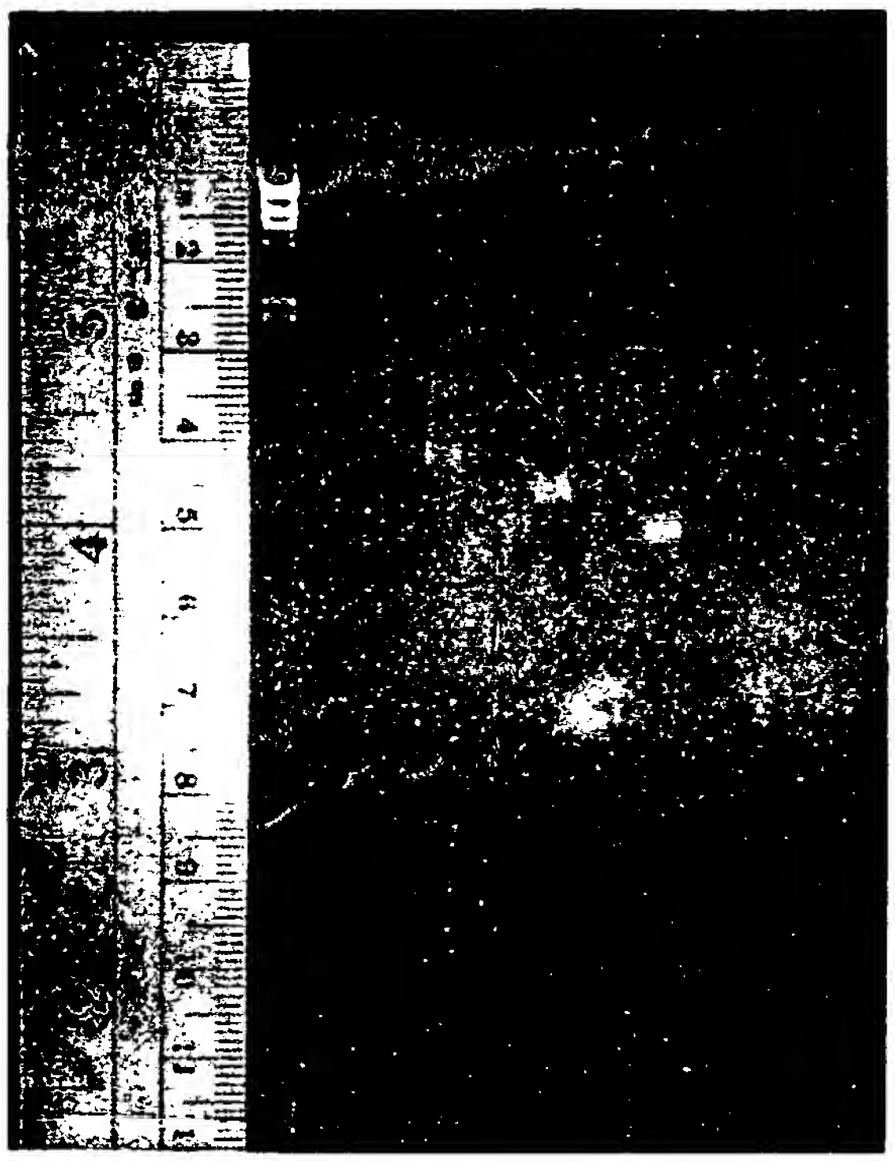
30									
31									



11/7/7

	1	2	Size of wide insert	RE Sites	5	Fragment	Enzyme	Amount of insert Cut	percent of insert cut out of gel	DNA expec = 60% recov
1										
2	NT.1b		4.3Kb	HindIII		0.85Kb	Dde I	~6µg	20%	600ng
3										
4	12.1(1.1)		3.0Kb	H3/SSt	(from 1.1Kb)	0.45Kb	Hha I	~3µg	41%	650ng
5										
6	20		1.25Kb	H3/SSt		0.65Kb	Rsa I	~6µg	52%	1.5µg
7										
8			NT1b - blunT 100ng ē Klenow							
9			12.1(1.1) "	" 50ng "		T4 Pol "		10d Rxn		
10			20 "	" 75ng ē	"	"		1d enzyme		
11								1d 20mM dNTPs		
12								1d 10x buffer		
13								1hr 37°		
14								-After- 65°x10'		
15								-add 5d 7.5M NH4Ac		
16								-add 35d etk.		
17								-ppt.		
18										
19										
20								Concentrations (½ λ)		Amount for Blunt
21										
22								NT.1b	0.85Kb 30ng/λ	3.4λ
23								20	0.65Kb 20ng/λ	3.75λ
24								12.1(1.1)	0.45Kb 40ng/λ	1.25λ
25										
26								NT.1b	20	12.1
27							DNA	3.4λ	3.75λ	1.25
28							Enz.	1, Klenow	1, T4	1 T4
29							dNTP's	1	1	1
30							10x Buff	1	1	1
31							H_2O	3.6	3.25	5.75



[illegible]

Joel Ranier

10	20	30	40	50	60
TCAGCTTGAG	ATGCTCTCAC	CTTTTCCTAA	TTTCAGAATC	CACAGTAATC	TGCCTCTTCT
70	80	90	100	110	120
TTTCGGCAGG	TGGTGGTGGA	AGTTTC[illegible]	GAGCATGCTT	TACTAGGATC	TGTTCCCTTG
130	140	150	160	170	180
TGGTCAGCGT	AGTTACTGCT	TCCATTACAG	TTGTCTGTGT	TAC[illegible]	[illegible]
190	200	210	220	230	240
TGACAGCCTG	TGAAATCTGT	GAGAAGTATT	GAAACAGAGG	TCAGACATTG	TTCGAAAGAC
250	260	270	280	290	300
TTCAGTAAAG	ACTGCTTGGA	GTGGCAAAGG	AAAATAATGA	GAAACTGCTC	TTGCTTGTTG
310	320	330	340	350	360
GTAGATTGAC	CTTCAGATCA	AAATAGCACC	ATATTGAAGC	CAAATAAAAT	TGTTTTATCT
370	380	390	400	410	420
GACTTAAGTT	TGGTTCACTG	AAATAGCAAG	GCAAAGTAAT	CTCAACATCG	AAAGTCAATT

TA

Preliminary Sequencing of 2 clones from 3' 1/11/8

	1	2	3	4	5	6	7	8	9
1									
2	Will sequence		12.1	IVa	0.45	HhaI into PTZ			
3			NT.3	IVa	0.5	DdeI into PTZ redo (T lane not Clear			
4									
5	1/12/8		12.1	VIb	DH5α F'				
6			47.40.2	IVa	0.2	HincII/PvuII	DH5α F'		
7			NT.3	IVa	again 0.5	DdeI PTZ,	DH5α F'		
8			NT.3	Ib	0.5	DdeI	PTZ	DH5α F'	
9									
10									
11									
12									
13									
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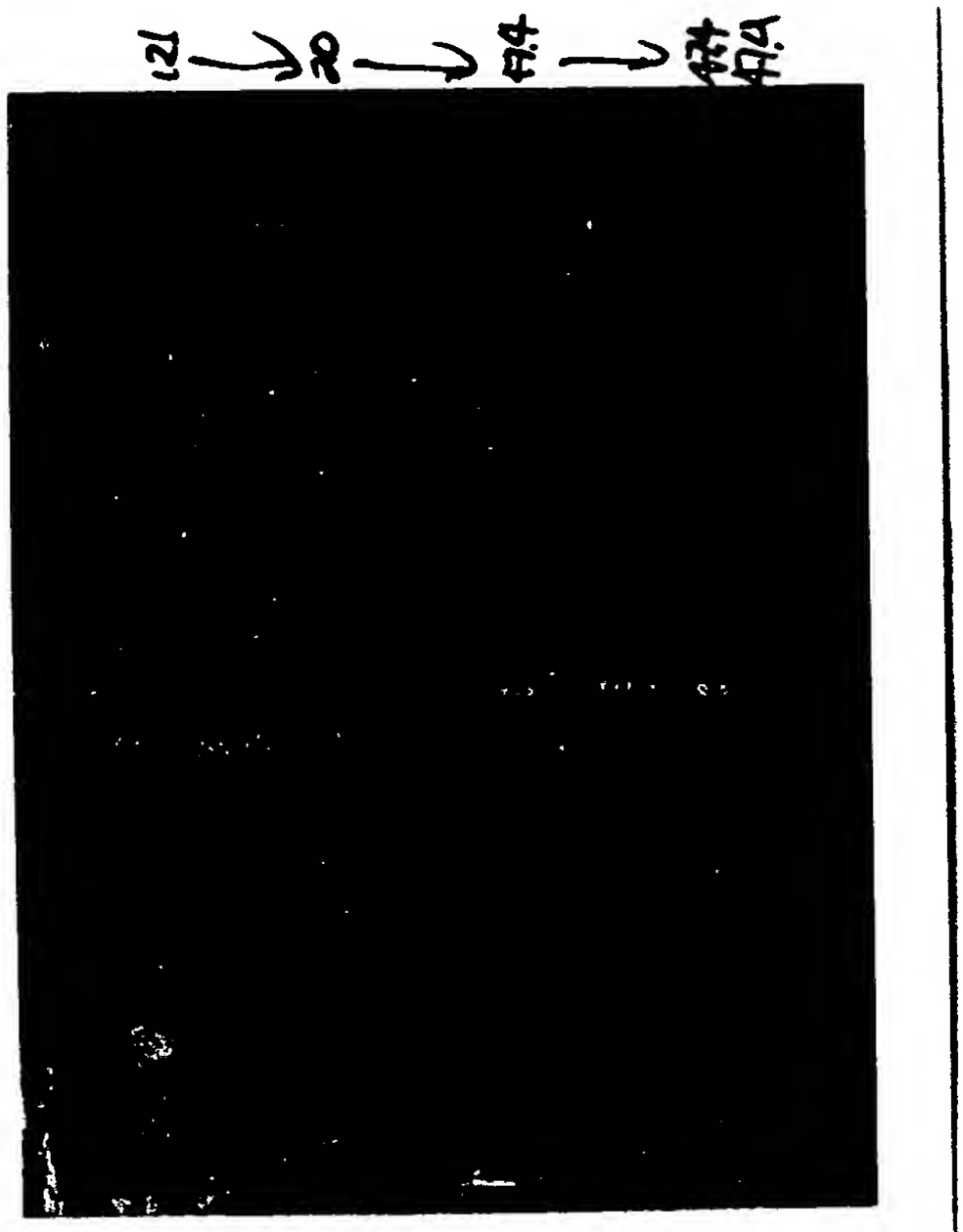
29									
30									
31									

Mini-Preps 12.1 (.45Kb) 20 (.65Kb), 47.4 (.6Kb) 11/17/7

	1	2	3	4	5	6	7	8	9	
.45Kb	12.1	VI	(blue)							
2	12.1	VII		Soln.	1	1.5ml				
3	12.1	VIII			2	3ml				
4	12.1	IX			3	2ml				
.65Kb	20	VI	(blue)							
6	20	VII								
7	20	VIII								
8	20	IX								
.6Kb	47.4	III	(blue)							
10	47.4	VIII	"							
11	47.4	IX	"							
12	47.4	X	"							
13	47.4	XI	white							
14										

15 Gel of Mini-Preps (inserts ran off gel) over

	DNA	(µl)	10xBuff (µl)		Enz.	(µl)	Sper.	RNase	TE	
.45Kb 1	12.1	10	2	2	H3/Sst	1+1	1	2	3	
2	12.1	10	2	2	"	"	"	2	"	Mix
3	12.1	10	2	2	"	"	"	"	"	24 TE
4	12.1	10	2	2	"	"	"	"	"	16 Buff
.65Kb 5	20	10	2	2	"	"	"	"	"	16 RNase
6	20	10	2	2	"	"	"	"	"	8 Sper
7	20	10	2	2	"	"	"	"	"	8 H3/
8	20	5	2	2	"	"	"	"	8	8 Sst
.6Kb 9	47.4	5	2	2	H3	1	"	"	9	
10	47.4	5	2	2	"	1	"	"	9	
11	47.4	5	2	2	"	1	"	"	9	
12	47.4	5	2	2	"	1	"	"	9	
13	47.4	5	2	2	"	1	"	"	9	
30										
31										



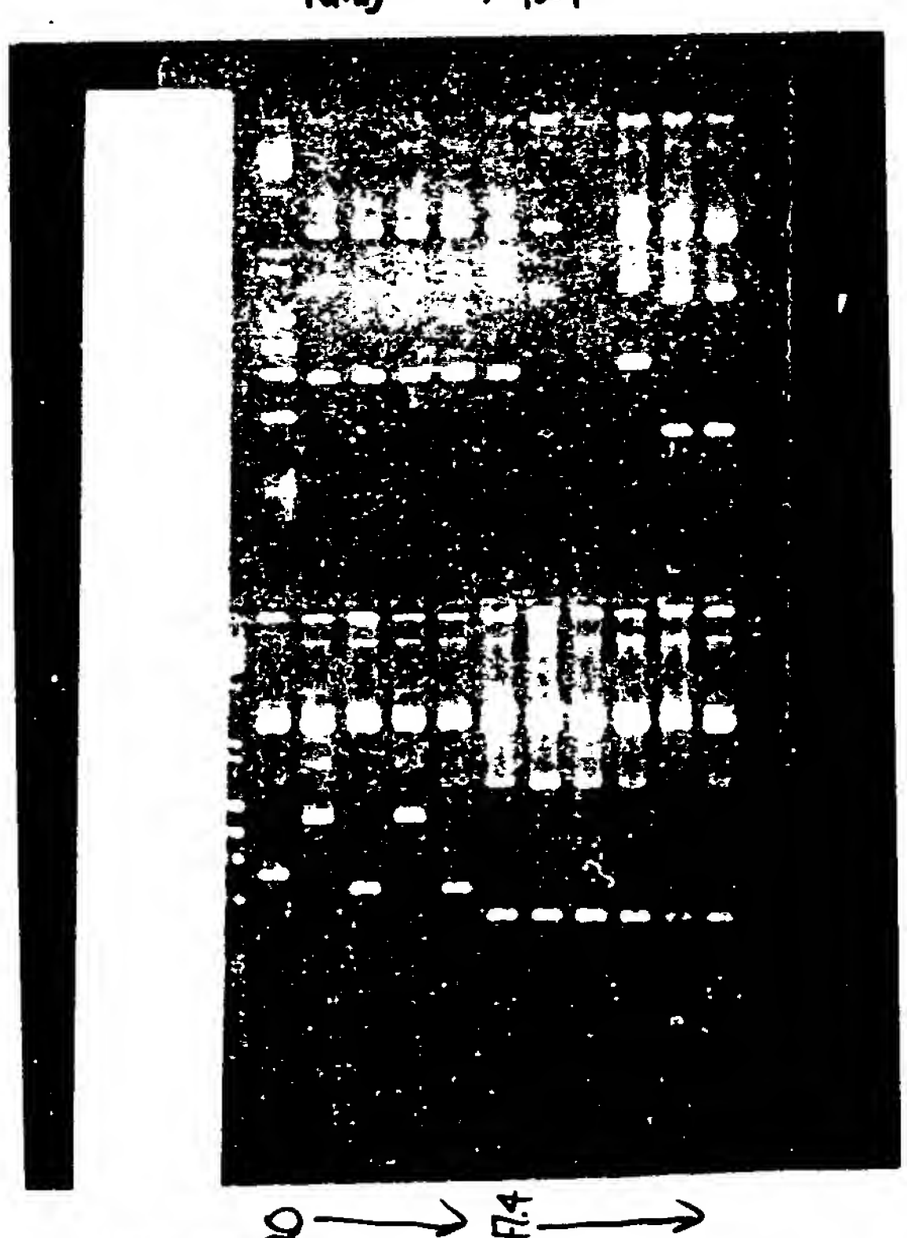
Jeff ran it too far

Joel Ramirez

Mini-Prep Gel NT.1b(0.85), 12.1(0.45) 20(0.65) 47.4(0.6kb) 11/15/7

	DNA	(µl)	10xBufS	(µl)	Enz	(µl)	Spermdine	RNAseA	TE	Total
1	λ-ΦX	20								
√(1) 2	NT.1b (0.85)	2	2	2	H3/SST	1+1	1	2	11	
√2 3	NT.1b	2	2	2	"	"	1	2	11	
√3 4	NT.1b	2	2	2	"	"	1	2	11	
√4 5	NT.1b	2	2	2	"	"	1	2	11	
√5 6	NT.1b	2	2	2	"	"	1	2	11	
6 7	12.1 (0.45)	5	"	"	"				8	
7 8	12.1	5	"	"	"				8	
8 9	12.1	5	"	"	"				8	
√9 10	12.1	5	"	"	"				8	
√10 11	12.1	5	"	"	"				8	
11 12	20 (.65)	5	"	"	"				8	
12 13	20	5	"	"	"				8	
√13 14	20	5	"	"	"				8	
14 15	20	5	"	"	"				8	
√15 16	20	5	"	"	"				8	
16 17	47.4	14	"	"	H3	1			—	
17 18	47.4	14	"	"	"	1				
18 19	47.4	14	"	"	"	1				
19 20	47.4	14	"	"	"	1				
20 21	47.4	14	"	"	"	1				
21 22	47.4	14	"	"	"	1				
23										
24										
25										
26										
27										
28										
29										
30										
31										

To do - pick more 47.4 0.6kbE/Bg (same blue)
" 12.1(.45), 20(.65)
for better chance of opp. orientations



Joel Ranier

2nd Gel of Mini-Preps 12.1(.45) 20(.65) 47.4(.6) 11/29/7

(prepped 11/27)

	1	2	3	4	5	6	7	8	9
1									
2	DNA	(µl)	10XBufC	(µl)	Enz.	(µl)	Sper.	RNaseA	TE
3	λ-ΦX	20							
✓1 4	12.1 (VI)	13	2	2	H3/SstI	1+1	1	2	—
✓2 5	12.1 (VII)	13							
✓3 6	12.1 (VIII)	13							
✓4 7	12.1 (IX)	13							
✓5 8	20 (VI)	13							
✓6 9	20 (VII)	13							
7 10	20 (VIII)	13							
8 11	20 (IX)	10							
9 12	47.4 (VII)	10			H3	2			3
10 13	47.4 (VIII)	10							
11 14	47.4 (IX)	10							
12 15	47.4 (X)	10							
13 16	47.4 (XI)	10							
17									
18	12.1	0.45KB	0.5?						
19	20	0.65KB							
20	47.4	C.6KB	0.55						
21									
22									
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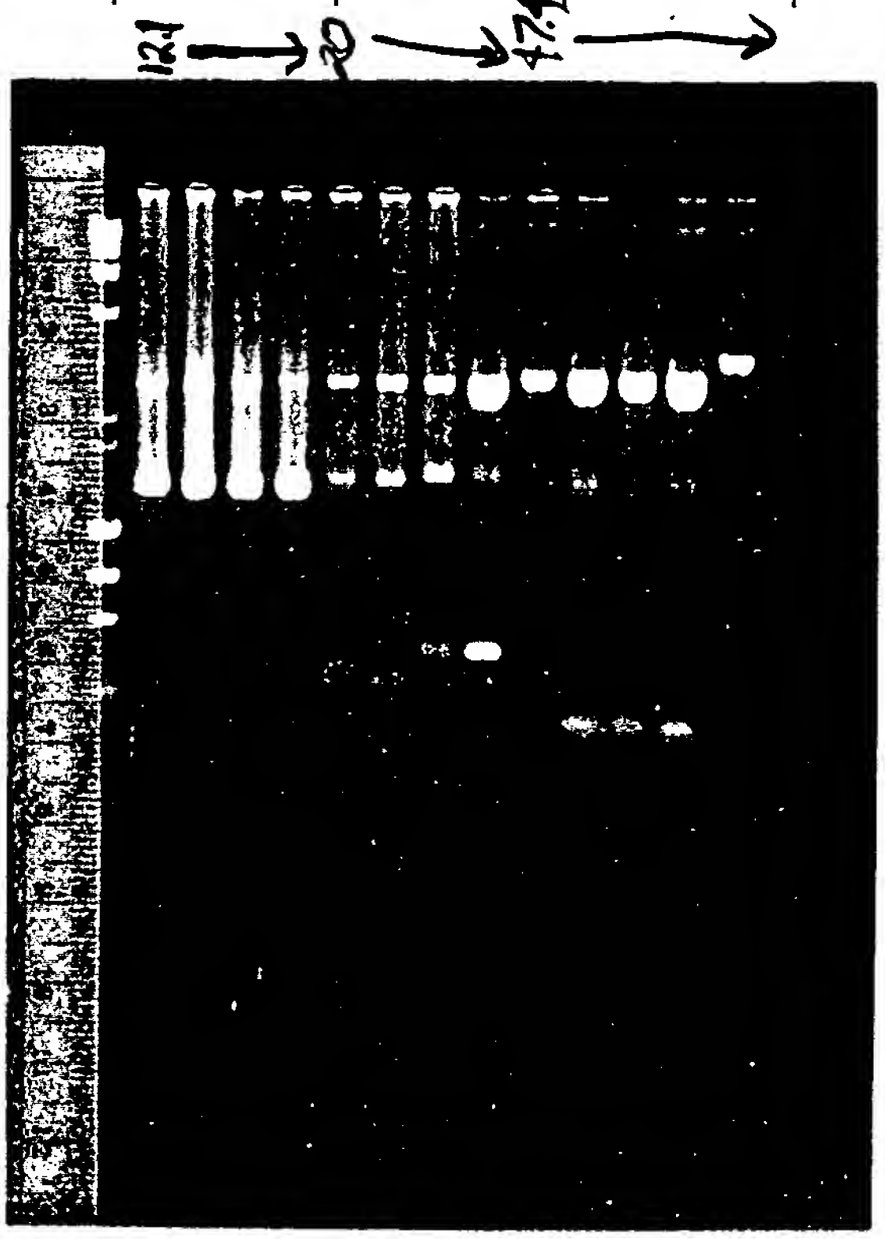
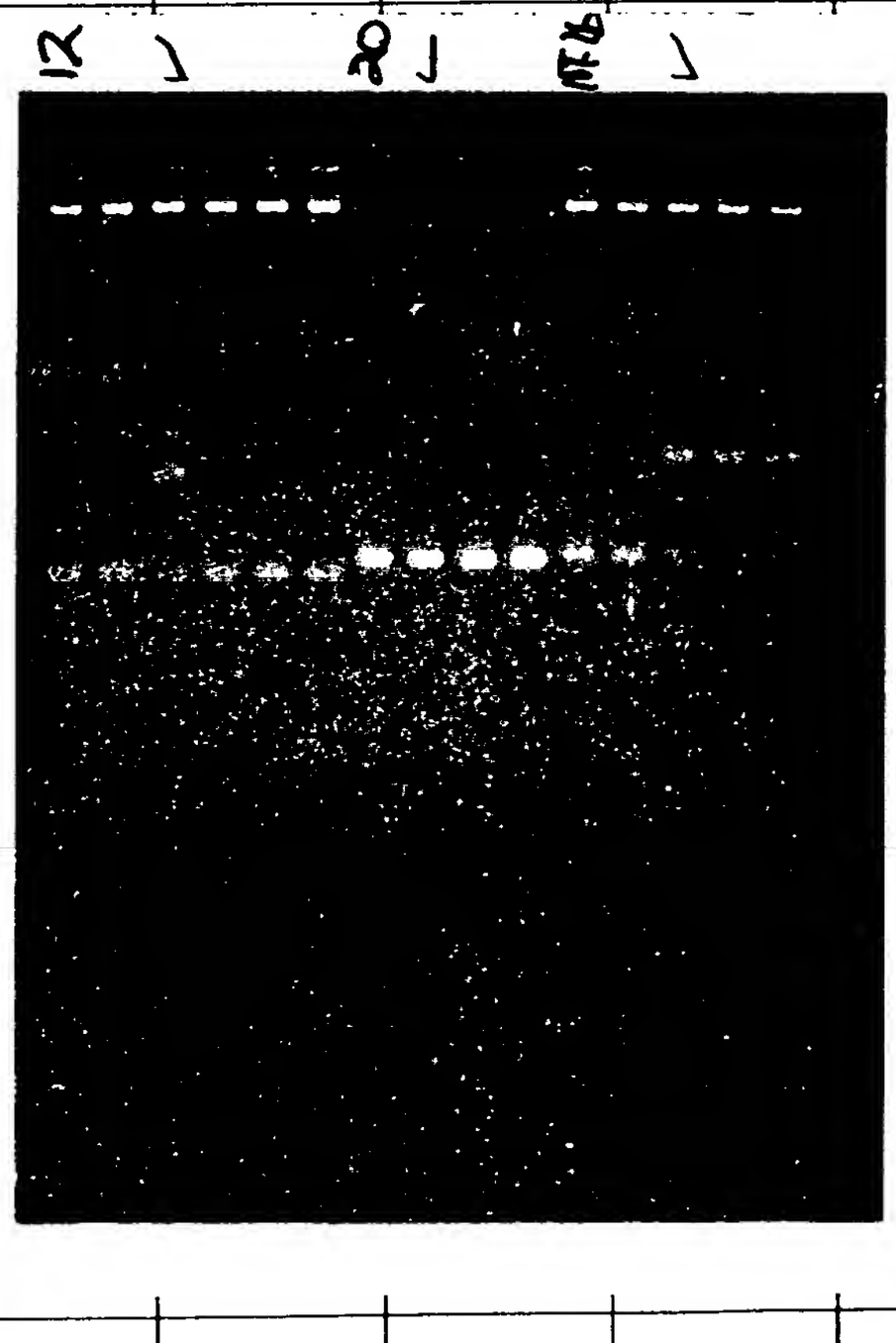
28									
29									
30									
31									



Joel Ranier

C-Tests on Clones 12.1(0.45), 20(0.66), + NT.1b(0.85) 12/11/7

	1	2	3	4	5 DH5αF'	6 TG-1	DH5αF'	9
1								
2	1ml of 3ml culture grown up to 0.5-0.8 OD							
3	Infected c Helper Phage and grown o/N							
4	Supernatant from tubes not to be used for purification							
5	used for C tests							
6								
7	✓ A 12 V	B 12 VII	✓ C 12 VI	D 12 IX	E 12 IV	F 12 VIII		
8								
9	20 µl	10	10	10	10	10		
10	A→	10	10	10	10	10		
11								
12	✓ G 20 III	H 20 V	I 20 VI	J1 20 III				
13								
14	20	10	10	10				
15	G→	10	10	10				
16								
17	✓ J2 NT.1b I	K NT.1b V	✓ L NT.1b II	M NT.1b IV	N NT.1b III			
18								
19	20	10	10	10	10			
20	J2→	10	10	10	10			
21								
22	65°C 1hr c 1x 2% SDS							
23				2.5x Dye				
24								
25	Vortex, spin 30 sec.							
26								
27	Run .9% gel							
28								
29								
30								
31								



Joel Ranier

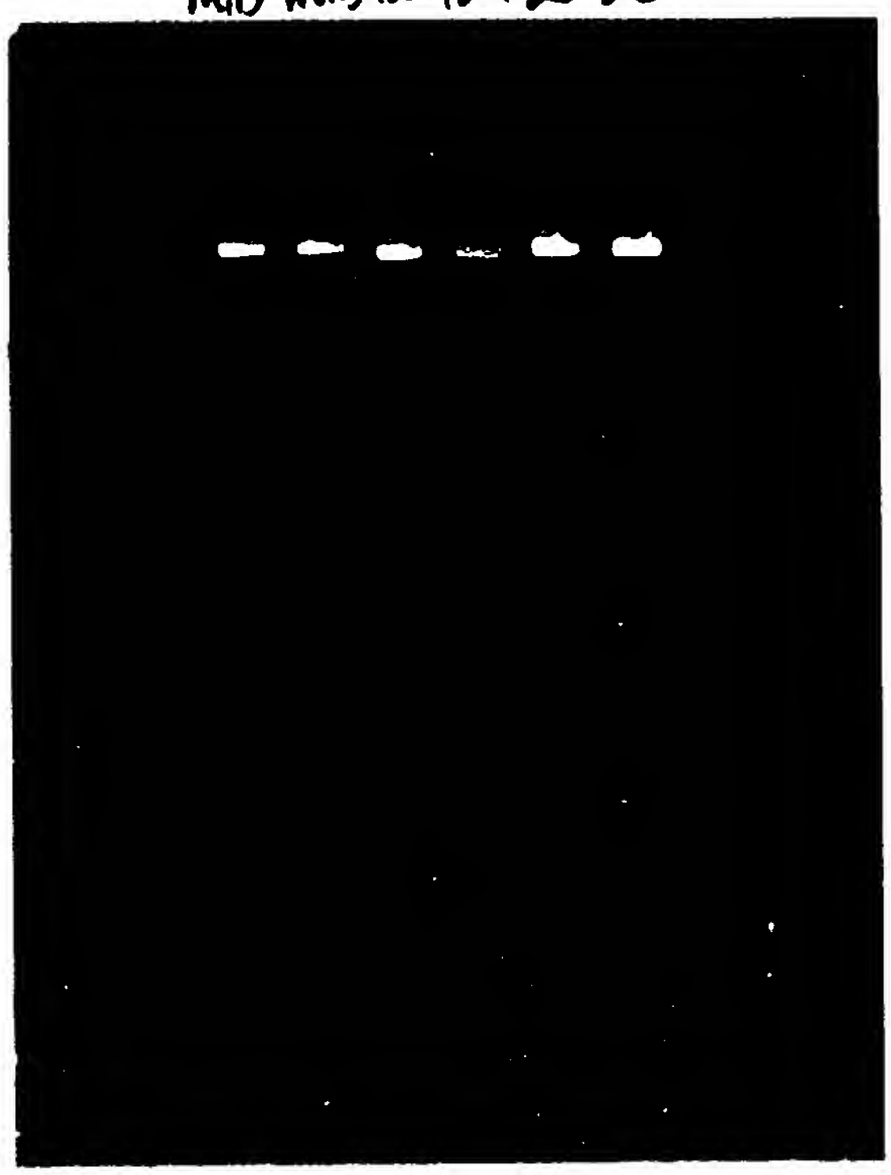
Purification of DNA for Sequencing 12/18/7

1)

NT.1b I & II
(opp. orientations)

12.1 V + VI
(opp. orientations)

20 VI + VII
(not opp.)



Joel Ravin

Screening of 4X Hu. Gen. Lib. (λ-zap) 11/2/87

Plated out ~ 46,000 plaques / 11 plates

Lifted onto hylon filters (2x)
Hybridized at 40% Formamide 12 hrs.
Washed 2x RT 5' 2xSSC
2x 60°C 20' 2xSSC
1x 45°C 15' 0.1xSSC

(11 - lost T. Agar
Condensaton on another date)

Labelling A7.4 0.6 KB E/Bg fragment

101589 x 975 ÷ 3 x 2 = 66 x 10^6 counts
68000/λ
650λ for 44 x 10^6 counts / 44 ml
880λ HS. DNA

F_1

A=101589.0(0.5%) B=101575.0(0.5%) C=000000.0(>20%)

F_2

A=031934.0(1.0%) B=031929.5(1.0%) C=000000.0(>20%)

* 18 Positives - Picked 10
ON, TW, TH, FR, FV, SX, SN, NN.1, NN.2, TN

Screening of Secondaries, Tertiaries 4x 11/10/87

1 2 Lib 3. vs 47.4 0.6KD E/Bg

Titer of 1° pk → 10 x 10⁶/ml
10 x 10³/λ 10,000/λ

Secondary screen pos. for all except TW FR
Will ~~re~~screen FR (more dense 1/100, 10λ) ē 3°

Titer of 2° pk → 5 x 10⁵/ml
500/λ

474 – 68000 counts/λ
want 5 x 10⁶/10 ml

80λ Probe
200λ H.S. DNA

Approximate titer of 90 clones from 4x 4lb. (to make amplified phage stock)

11/17/7

For 1500-2000 Φ replated 7λ (gave 9Φ), 100λ

	ON	100 x 100 x 100 = 1.0 x 10⁷/ml 1000/λ		
	IW	X		
300	IH	32 x 100 x 100 = 3.2 x 10⁷/ml 320/λ		6λ
200	FR	250 x 100 x 100 = 2.5 x 10⁷/ml 250/λ		8λ
200	FV	56 x 100 x 100 = 5.6 x 10⁷/ml 560/λ		3λ
200	SX	68 x 100 x 100 = 6.8 x 10⁵ 680/λ		3λ
100	SW	63 x 100 x 100 = 6.3 x 10⁵/ml 630/λ		3λ
91 ~~100~~	NN.1	200 x 100 x 100 20 x 10⁶/ml 2000/λ		10λ of 1/100, replated 15λ
1500	NN.2	55 x 100 x 100 = 5.5 x 10⁵, 550/λ		3λ
	IN			
1500		15 x 100 x 100 = 1.5 x 10⁵, 150/λ		10λ

Titers of Amp. Phage Stocks — 12/3/7

ON

$1/100$, 11λ — $200 \times 91.00 \times 100 = 1.82 \times 10^{6}$/ml

100,000 → ~~27λ~~ 60

IH

$1/10^{3}$, 18λ — $20 \times 55.56 \times 10^{3} = 1.10 \times 10^{6}$/ml

For 100,000 → ~~46.0λ~~ 100λ

FR

$1/10^{3}$, 53λ — $36 \times 18.87 \times 10^{3} = 6.8 \times 10^{5}$/ml

For 100,000 → ~~74λ~~ 150

FV

$1/10^{3}$, 20λ — $16 \times 50 \times 10^{3} = 8 \times 10^{5}$/ml

For 100,000 → ~~63λ~~ 150

SX

$1/10^{3}$, 20λ — $9 \times 50 \times 10^{3} = 4.5 \times 10^{5}$/ml

100,000 → 200λ

SN

$1/10^{3}$, 75λ — $6 \times 13.33 \times 10^{3} = 8 \times 10^{4}$/ml

To reamp — 15000 → take 19λ

Titers of Amp. Phage Stocks (con't) 12/3/7

1/10⁴, 10λ

NN1

12 x 55.56 x 10⁴ = 6.67 x 10⁶/ml

For 100,000 → 1/10, 20λ

NN2

1/10⁵, 40λ

8 x 25 x 10⁵ = 2 x 10⁷/ml

For 100,000 → 1/10, 10λ

TN

1/10⁵, 100λ

10 x 10 x 10⁵ = 1 x 10⁷/ml

For 100,000 → 5λ → 1/10, 15λ

ET.1

10³, 50

300 x 20 x 10³ = 6 x 10⁶/ml

For 100,000 → 1/10, 20λ

ET.2

10³, 15λ

475 x 66.67 x 10³ = 3.17 x 10⁷/ml

For 100,000 → 1/10, 5λ

Phage Lysates (474 0.5Kb clones + 63.1 1.0Kb (2)) 12/4/7

Lowest plate – 40,000 ø
Highest plate – just confluent

Should have aimed for 300,000 instead of 100,000
Don't do amplification ē plate ē less than 5000 ø

Eluting ē 10mls SM for lysate

→ Estimated vols. of phage lysate

ON →	12mls	stock 1mg/ml →	12µl	
TH →	11.0mls	→	11.0µl	
FR →	11.5mls	→	11.5µl	
FV →	~~10.0mls~~ 9.0	→	~~10.0µl~~ 9.0µl	
SX →	~~11.5mls~~ 10.5	→	10.5µl	
NN.1 →	~~11.5mls~~ 11.25	→	11.25µl	
NN.2 →	11.~~00~~mls	→	11.00µl	
FT.1 →	10mls	→	10µl	
FT.2 →	10.~~75~~mls 10.5	→	10.75µl 10.5	
TN →	11.~~75~~mls 11.5	→	11.75µl 11.5	

FT.1 10
FV 9.0
FT.2 10.5
NN.2 11.00
TH 11.0
FR 11.5
SX 10.5
NN.1 11.25
TN 11.5
ON 12

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O.D.'s 5λ → 200 of Phage lysate DNA 12/5/7

	260	260/280	conc	yield
ON	2.504	1.879	5.0 mg/ml	1.0 mg
TH	2.555	1.809	5.1 mg/ml	1.0 mg
FR	2.585	1.677	5.2 mg/ml	1.1 mg
FV	2.585	1.71	5.2 mg/ml	1.1 mg
SX	2.545	1.90	5.1 mg/ml	1.0 mg
NN.1	1.794	2.276	3.6 mg/ml	0.72 mg
NN.2	2.394	1.975	4.8 mg/ml	0.96 mg
TN	1.928	2.27	3.9 mg/ml	0.78 mg
FT.1	2.073	2.22	4.1 mg/ml	0.82 mg
FT.2	2.307	2.00	4.6 mg/ml	0.92 mg

47.4 Genomic Clone Fragments For ~~Blunt-ending~~ Ligating → pUC18 1/11/18

	Insert	Enzyme	Concentration	Yield
ON	4.36Kb	Eco	~25ng/λ	250ng
FV	3.0Kb	Eco	~10ng/λ	100ng
NN.1	3.8Kb	Eco	~7.5ng/λ	75ng
TN	~17Kb	Eco	~50ng/λ	500ng

Cut 100λ of each
ideally want to use 150ng
here use 5λ

pUC18 Eco Cip 200ng/λ
box 7 C7
dilute 1/10 → 2.5λ for 50ng
1/5 → 1.25

DNA	5λ	
10mM ATP	1λ	
pUC18	~~2.5~~	1.25λ
10x Lig	1λ	
T4 Lig	1λ	
H_2O	.75λ	
	10λ	

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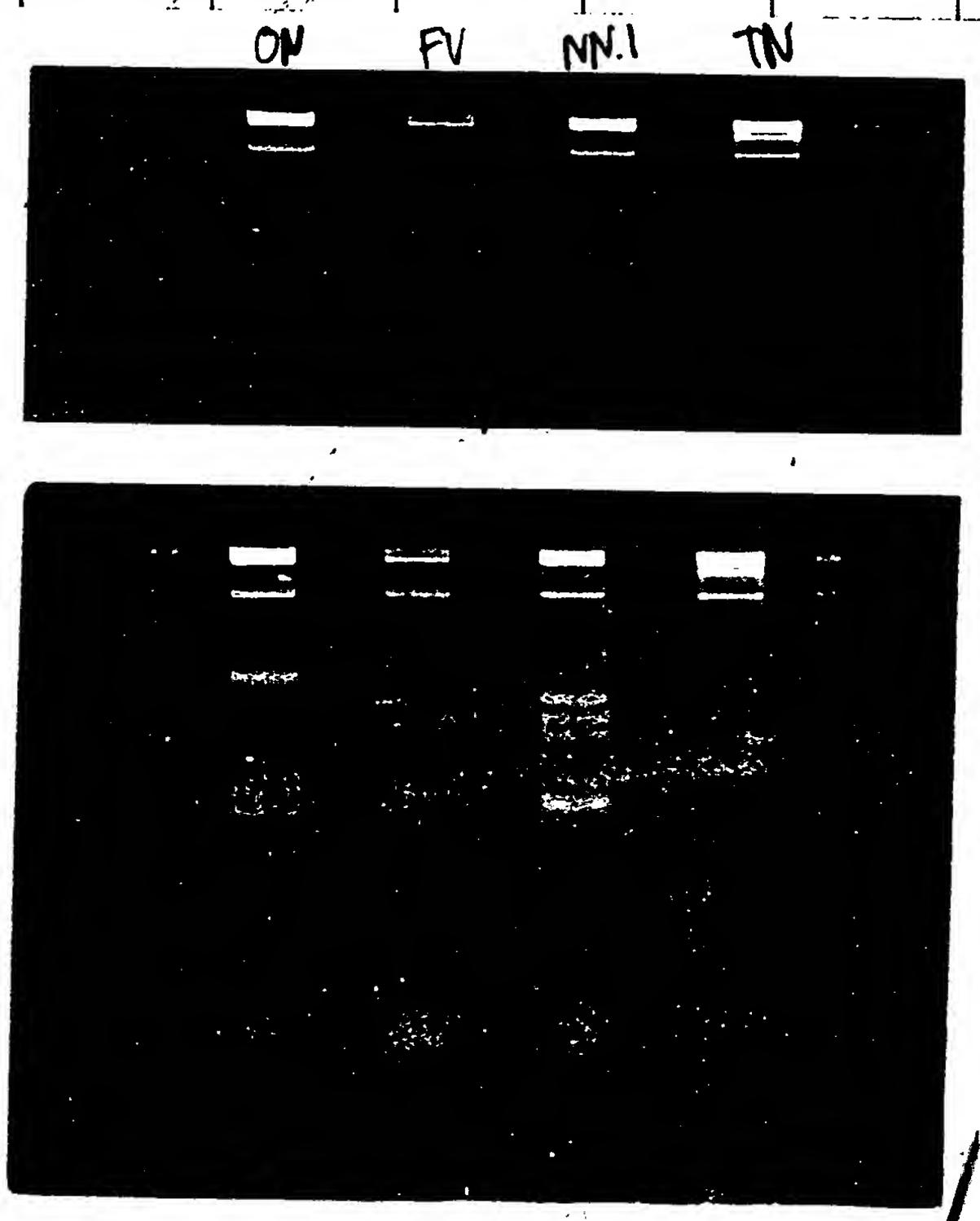
Prep Gel of 47.4 clones ON, FV, NN.1, TN 12/28/7

	(1) DNA	(2) (µl)	(3) 10X Buff	(4) Enzyme	(5) (µl)	(6) Ster.	(7) RNase A	(8) TE	(9) Tot
1	ØX-7	15							
2	ON	100	3, 20	EcoRI	20	8	5	47	200
3	4.36Kb								
4	FV	100	3	"	"	"	"		200
5	3.0Kb								
6	NN.1	100	3	"	"	"	"		200
7	3.8Kb								
8	TN	100	3	"	"	"	"		200
9	2.7Kb								



Mini-Prep of 47.4 clones ON, FV, NN, TN 1/19/8

	1	2	3 in pUC18	5	6	7	8	9
1								
2	ON	I						
3	(4.36)	II		For 5ml soln 2				
4		III						
5		IV ✓		10N NaOH	100 µl			
6		V		25% SDS	200 µl			
7		VI		H_2O	4700			
8	FV	I ✓			5ml			
9	(3.0)	II						
10		III						
11		IV						
12		V						
13		VI						
14	NN, 1	I						Mix (23)
15	(3.8)	II						10x '3' 46
16		III ✓						Sper. 46
17		IV						RNaseH 23
18		V						TE 184
19		VI						299
20	TN	I ✓						Add 37
21	(~17)	II X						DNA 5λ
22		III						Eco 2λ
23		IV						
24		V						
25		VI						
26								
27								
28	Fragments ligated into pUC18 Eco-cip							
29	Cut E Eco							
30	2.5 hr. digest							
31								

ON → FV →

NN.1 → TN →

Large Scale Prep of 47.4 0.6Kb E/Bg Subclones 1/25/8

Lg. prep of
ON (IV) 4.3L
FV (I) 3.0
NN.1 (II.) 3.8
TN (I) ~17

ON - 33.5 5.15g NH_4Ac
FV - 33.5 "
NN.1 31.5ml after remove
TN 35.5ml supe before NH_4Ac 4.85g NH_4Ac ⇐ 5.47g NH_4Ac

ON FV - 16.75 ml 7.5M NH_4Ac
NN.1 15.75 ml "
TN 17.75 ml

ON 46
FV 48
NN.1 46
TN 57

After dialysis For .1M NaCl

ON.	5.75	115 µl of 5M	3ml	18
FV	5.75	125 µl	"	18
NN.1	6.25	125 µl	3.25 ml	19.5
TN	3.75	75 µl	1.9 ml	11.5

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Concentration of Lg. Preps ON, FV, NN.1, TN 1/31/8

(A7.4 clones)

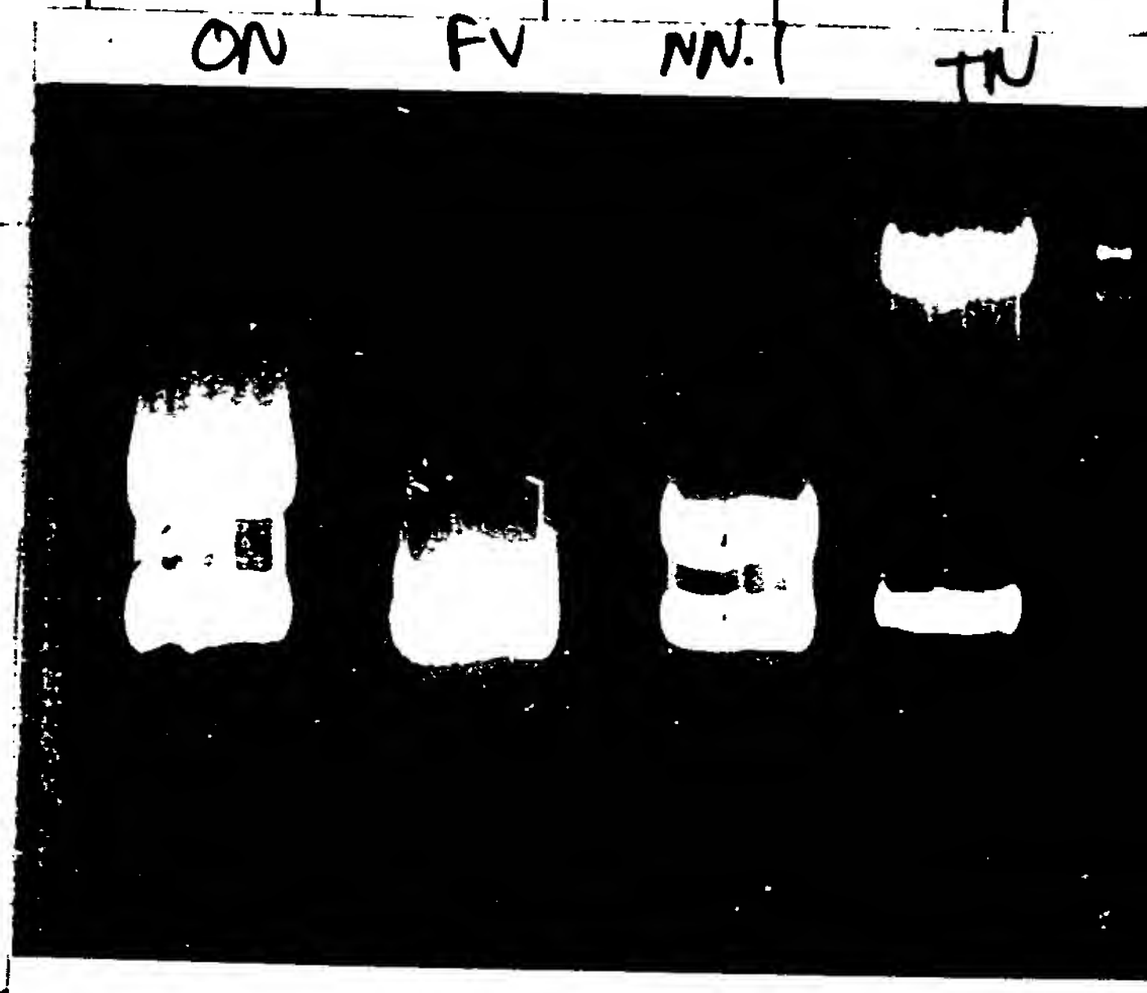
	1	2	A260		A260/A280		7 Yield	8	9 Conc
1									
2									
3	ON		2.492		1.674		1.25mg		2.5mg/ml
4	4.36								
5	FV		2.18		1.91		1.10mg		2.2mg/ml
6	3.0								
7	NN.1		.440		2.65		220µg		490µg/ml
8	3.8								
9	TN		1.80		2.01		900µg		3.6mg/ml
10	~17								
11	ON, FV	NN.1	10λ → 200						
12	TN		5λ → 200						
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									
31									

Gel Purify

Excision of Inserts from 47.4 clones

2/1/88

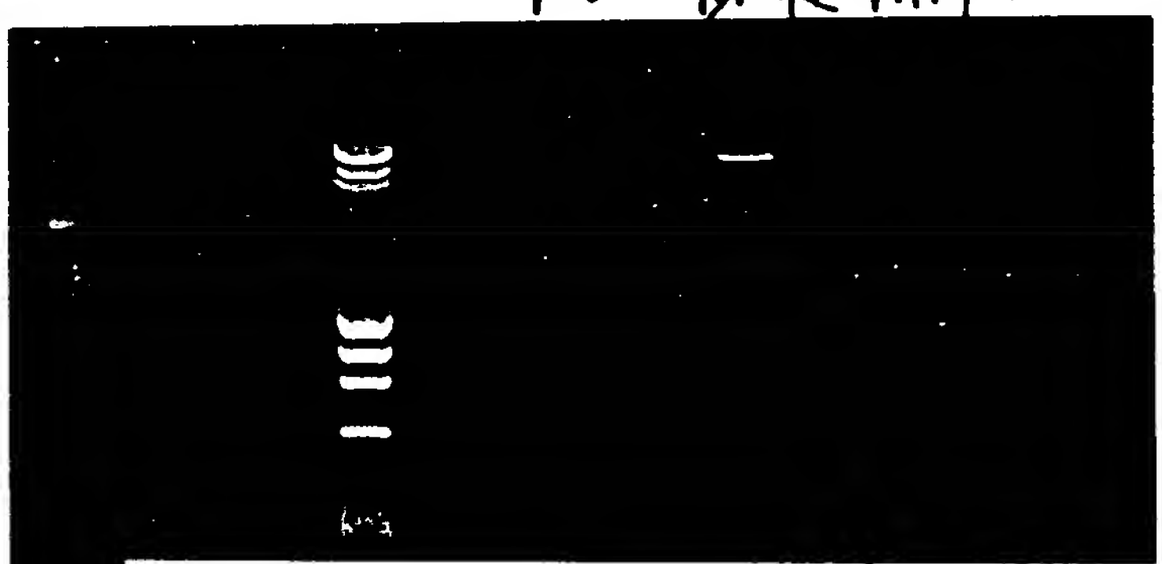
	1	2	3 Insert	4 Conc	5 Conc of Insert	6 % Insert	7 For 10ug Insert	8 RE sites	9
1									
2	ON		4.36	2.5mg/ml	1.5mg/ml	(60%)	6.7µl	EcoRI	
3	(4.36)								
4									
5	FV		3.0	2.2mg/ml	1.2mg/ml	(50.8%)	9.1µl	EcoRI	
6	(3.0)								
7									
8	NN.1		3.8	440µg/ml	250µg/ml	(56.7%)	40µl	EcoRI	
9	(3.8)								
10									
11	TN		~17	3.6mg/ml	3mg/ml	(85.4%)	3.2µl	EcoRI	
12	(~17)								
13									
14									
15	Cloned into EcoRI site pUC18								
16									
17	Mix								
18									
19	10x'3'	10	x4	40					
20	Sper	4	x4	16					
21	RNaseA	2	x4	8					
22	H_2O	24	x4	96					
23		ON	FV	NN.1	TN				
24	DNA	14	18	50	7				
25	H_2O	36	32	—	43				
26	Mix	40	40	40	40				
27	Enz	10	10	10	10				
28	(Eco)								
29	Cut Appox. 20µg DNA								
30									
31									



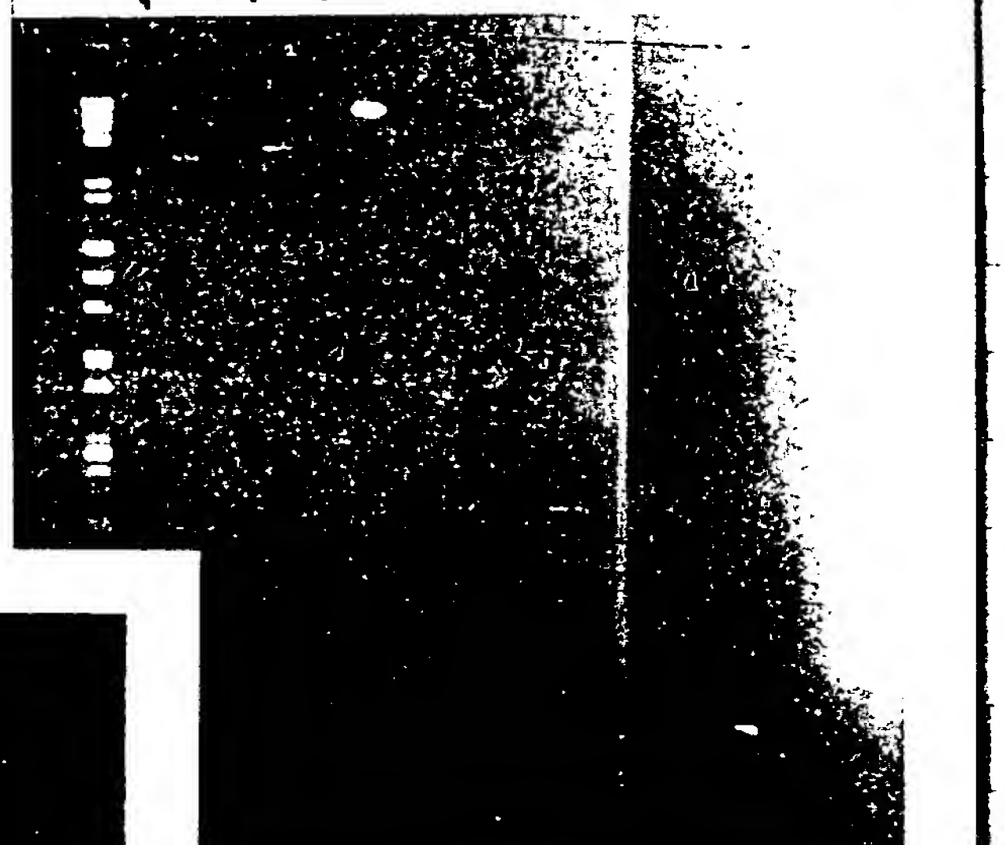
Concentration of NN.1, TN, FV 2/12/8

	1	2	3	Conc	5	Yield	7	8	9
1	Loaded	0.5λ							
2	FV	(3.0)		50ng/λ	(40λ)	~~100ng~~	2μg		
3									
4	NN.1	(3.8)		~~50~~ng/λ	(25λ)	~~250ng~~	1.25μg		
5									
6	TN	(~17kb)		100ng/λ	(25λ)	25μg			
7				(after gel 3/2		looks	like 50ng/λ)		
8						~1μg	remaining		
9									
10									
11									
12									
13									

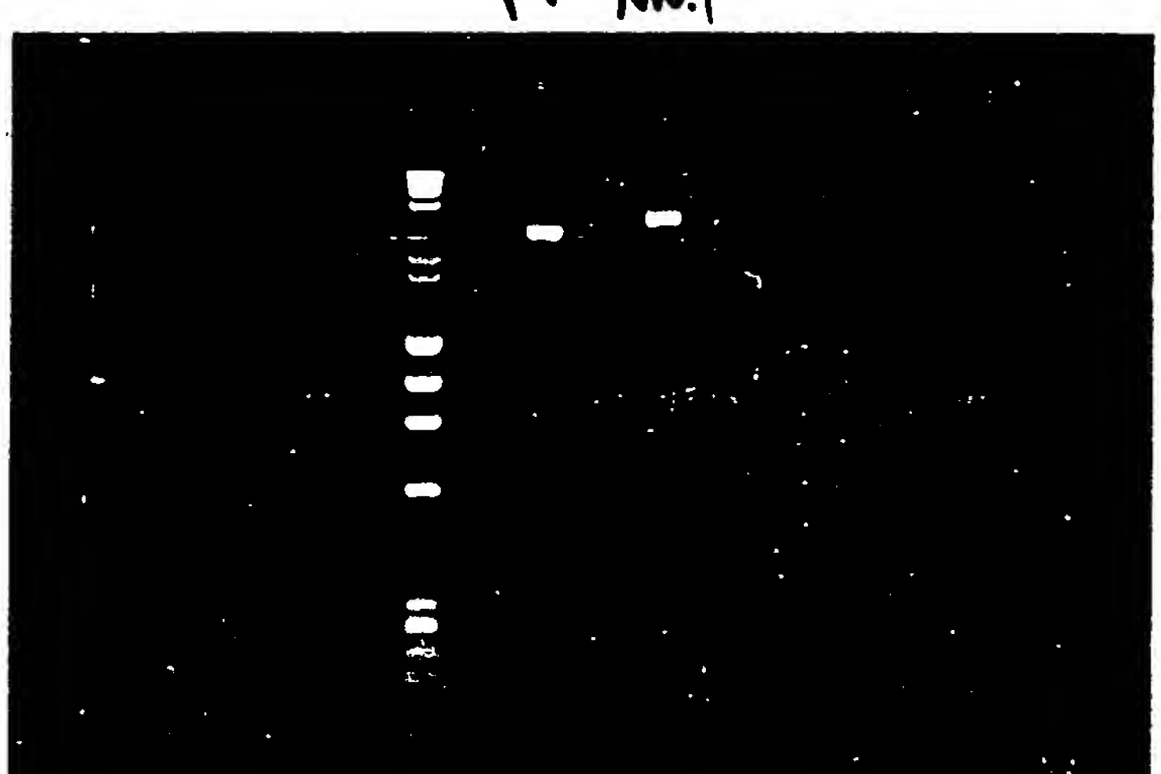
FV TN NN.1



FV NN TN (W/100C)



FV NN.1



← Relocation of FV NN.1
.5λ of 50λ
200ng/λ

26	
27	
28	
29	
30	
31	

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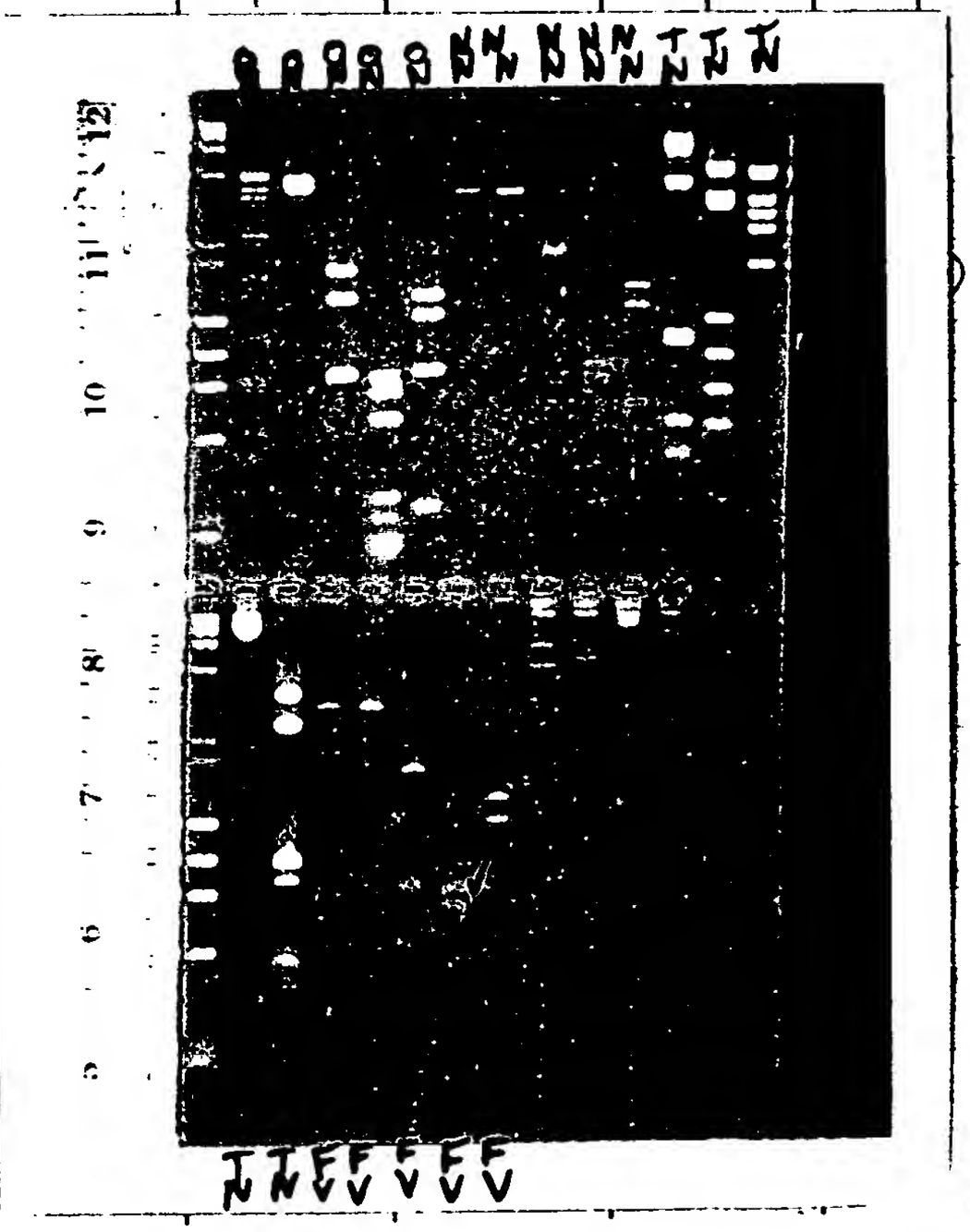
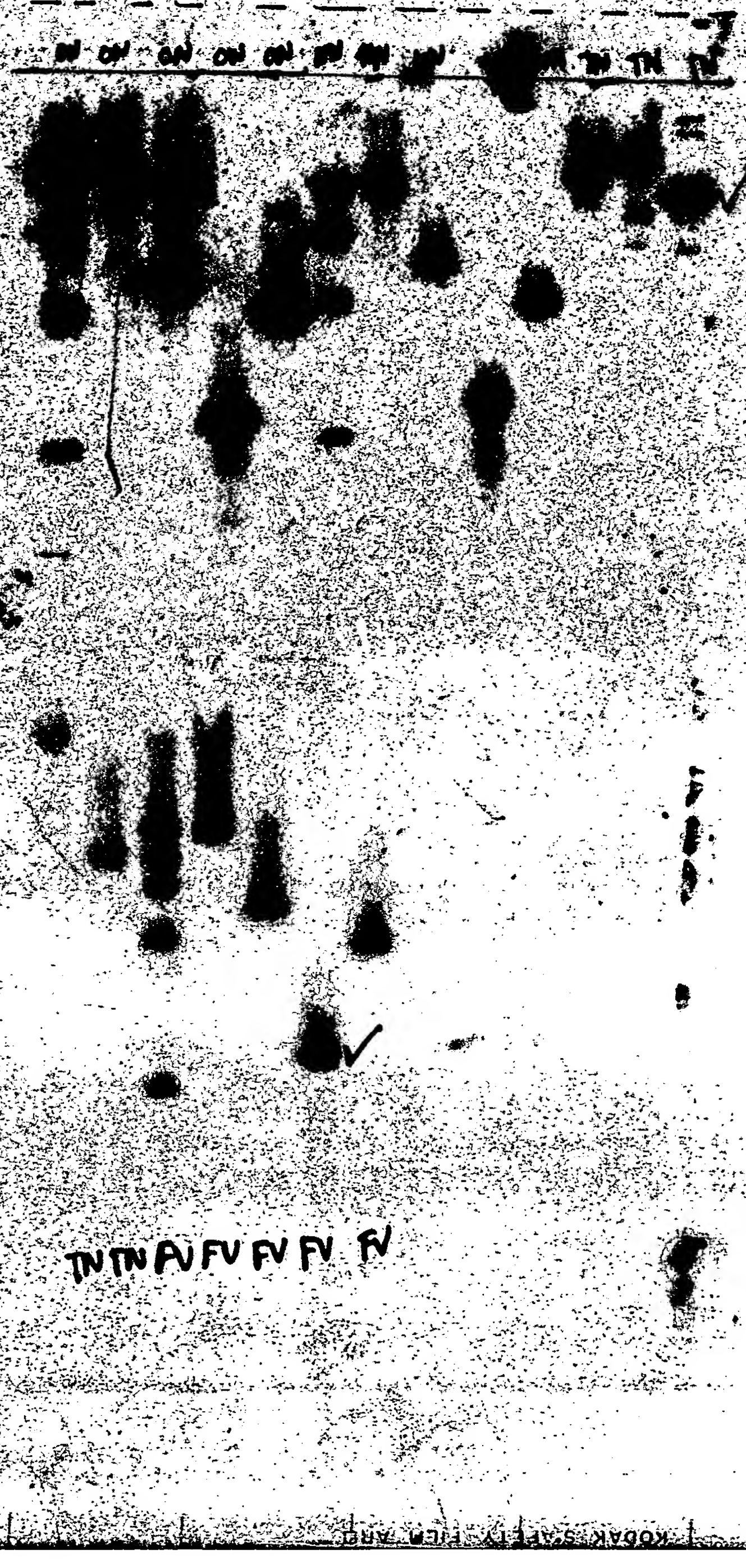
Gel of 47.4 subcloned fragments c 4 base cutters 2/28/8

	1	2 + TN	3 & 6 base cutters 4	5	6	7	8	9	
1									
2	DNA	(µl)	10X Buff	(µl)	Enzyme	(µl)	Sper	RNase	TE	TOT
3									
1	ON	1.5	1	2	RsaI	1	1	1	13.5	20
2	ON	1.5	2	2	HhaI	1	1	1	↓	↓
3	ON	1.5	2	2	TaqI	1	1	1		
4	ON	1.5	2	2	DdeI	1	1	1		
5	ON.D	1.5	3	2	BstNI	1	1	1	↓	
6	NN.1	5	1	2	RsaI	1	1	1	10	20
7	NN.1	5	2	2	HhaI	1	1	1	↓	↓
8	NN.1	5	2	2	TaqI	1	1	1		
9	NN.1	5	2	2	DdeI	1	1	1		
10	NN.1	5	3	2	BstNI	1	1	1		
11	TN	3	2	2	HindIII	1	1	1	12	20
12	TN	3	2	2	PstI	1	1	1	↓	↓
13	TN	3	3	2	BglII	1	1	1		
14	TN	3	3	2	BamHI	1	1	1		
15	TN	3	2	2	H3+PstI	1	1	1		
19		250 ng								
19	FV	8	1	2	RsaI	1	1	1		
17	FV	8	2	2	HhaI	1	1	1		
18	FV	8	2	2	TaqI	1	1	1		
19	FV.	8	2	2	DdeI	1	1	1		
20	FV	8	3	2	BstNI	1	1	1		
25										
26										
27										
28										
29										
30										
31										



479 clones
vs. CDNA
(OLwb FBs)
1hr exp
-70°C
0.1XSSC, 65°C
2/25/18



TN TN FV FV FV FV FV

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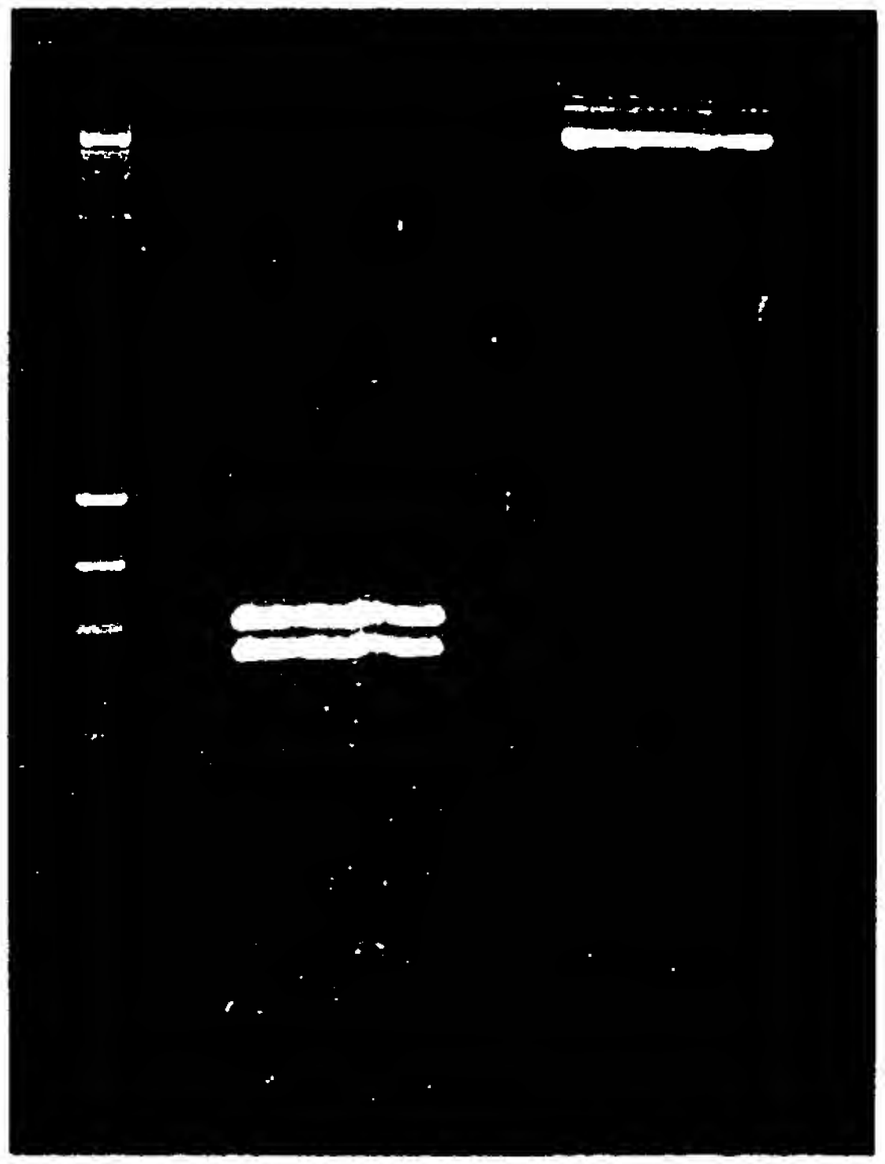
2/28/18

	1	(µl)	10X	(µl)	5	6	Sp	RNase	TE	
1	FV	40	2	10	DdeI	10	48	53	37	100
2										
3	12.1	100	2	20	HindIII	20	7	5	48	20
4	Phage Lysate									
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16					0.9 DdeI →					
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										

FV 12.1



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C-test (2nd) on FV(0.9), TN(3.0) 3/19/18

	R	S	T
	FV(0.9) 3	FV(0.9) 4	FV(0.9) 5
	20	10	10
R→		10	10

	U	V	W
	TN(3.0) 5	TN(3.0) 3	TN(3.0) 4
	20	10	10
V→		10	10

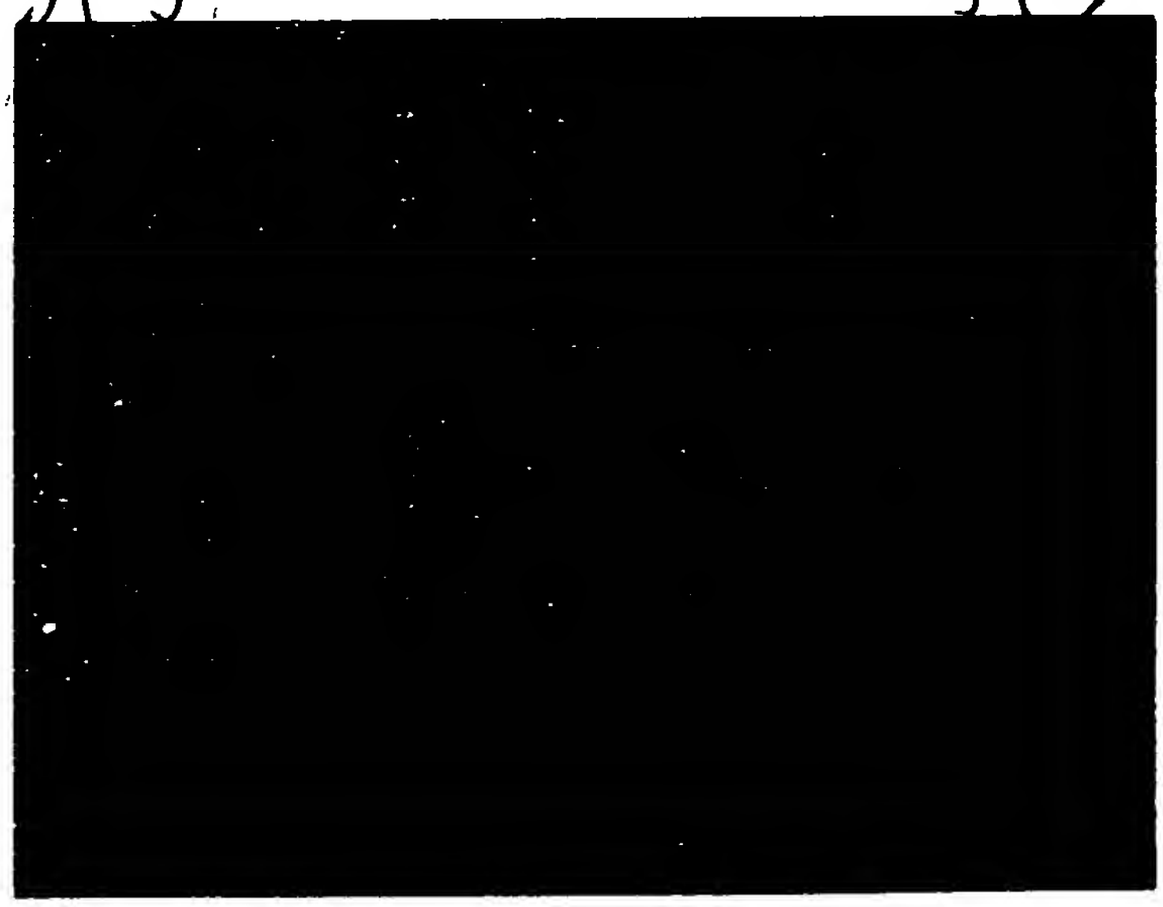
6↗ 2% SDS

15↗ Dye

Add 3.5↗

FV(0.9) 3A 5

TN(3.0) 5A 3



Negative

Ligation of FV .9Kb (DdeI), FV .5Kb (H3), 12.1 1.5Kb (H3) 3/5/8

1									
2	FV	DdeI	0.9Kb	(Blunt-ended)					
3									
4	DNA		5λ						
5	Vector	(PTZ19R HincII)	.5λ	(50ng)					
6	(10x Lig		1λ						
7	ATP)		1λ		Didn't seem to work				
8	T4		1λ						
9	H_2O		1.5λ						
10			10λ						
11	FV	0.5 H3							
12									
13	DNA		6.5λ						
14	Vector	(PTZ19R H3)	1λ						
15	10x Lig.		1λ						
16	ATP)		1λ						
17	T4 Lig		.5λ						
18			10λ						
19									
20	12.1	1.5 H3	(after reprecip)		(3/6/8)				
21									
22	DNA		5λ						
23	Vector	(PTZ19R H3)	1λ						
24	10x Lig		1λ						
25	ATP		1λ						
26	H_2O		1.5λ						
27			10λ						
28									
29									
30									
31									

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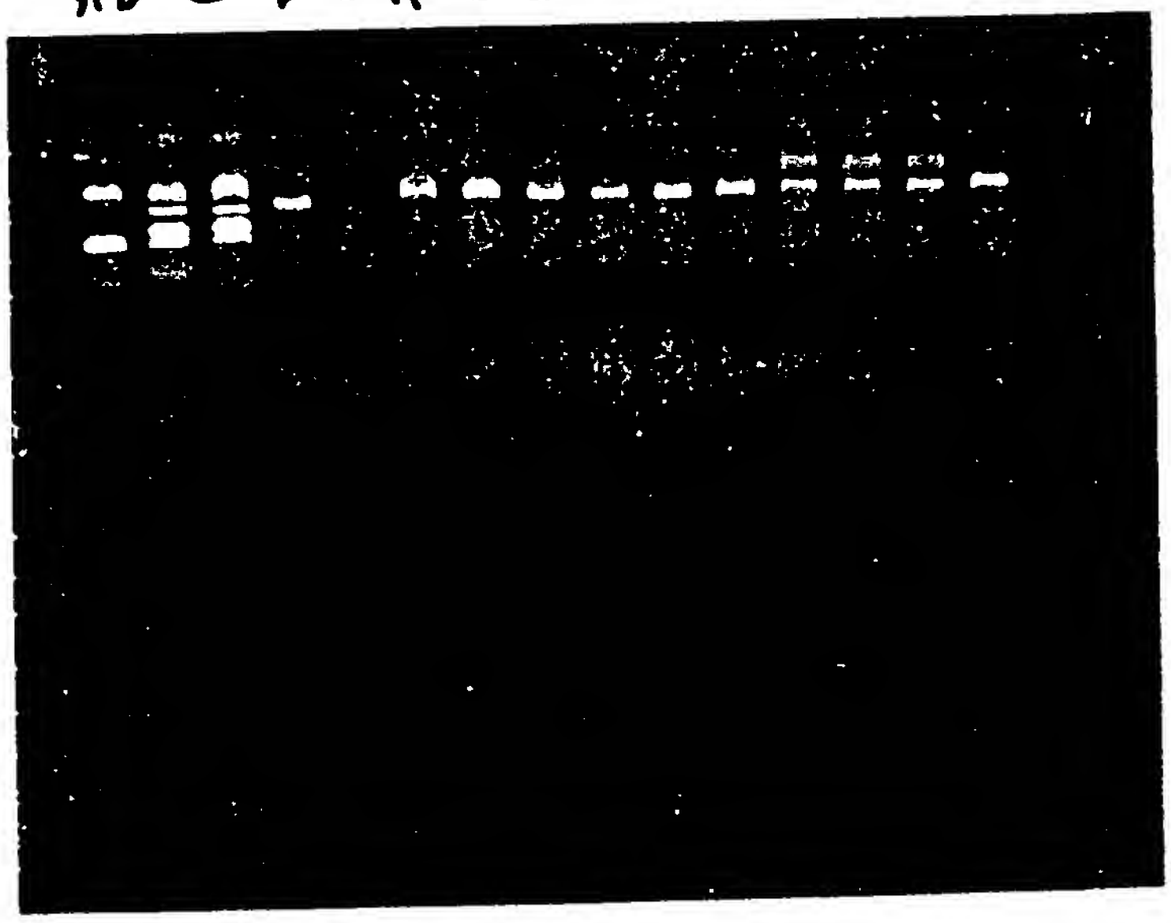
C-tests FV.5H3, FV.9DdeI, TN3.0Bg II 3/13/8

	1	2	3	4	5	6	7	8	9
1									
2	A	B	C		D	E			
3	FV(.5)	FV(.5)	FV(.5)		FV(.5)	FV(.5)			
4	III	IV	VI		IV	III			
5	2µl	10	10		2µl	10			
6	A→	10	10		D→	10			
7									
8	F ✓	G	H	I	J ✓	K ✓			
9	FV(.9)	FV(.9)	FV(.9)	FV(.9)	FV(.9)	FV(.9)			
10	1	2	3	4	5	6			
11	2µl	10	10	10	10	10			
12	1→	10	10	10	10	10			
13									
14	L ✓	M	N ✓	O	P	Q			
15	TN3.0	TN3.0	TN3.0	TN3.0	TN3.0	TN3.0			
16	1	2	3	4	5	6			
17	2µl	10	10	10	10	10			
18	1→	10	10	10	10	10			
19					FV.9H3 III IV VI ✓				
20	Add	3.5λ	of Mix				J ✓	K ✓	L ✓
21			18λ						
22			45λ						
23	vortex, cfg		65°C 1h						
24									
25	Run 0.9% gel								
26									
27									
28									
29									
30									
31									

A B C D E F G H I J K L M N O P Q



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Purified DNA TN(3.0), FV(0.9), FV(0.5) 3/13/18

17

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Sequencing FV.5 Ib, IIb 47.4 XIV, 12.1 3/22/8

FV(5) - re-transformed mini-prep DNA Picked white colony (DH5αF') Ib and blue colony IIb
PTZ19R H3 (C-test strange)
*Still saw shadow bands, but ~~was~~ was able to obtain enough data to make oligo's

12.1(1.5) - portion of XD-1 Clone (87.15 region)
W/O Bam polymorphism
PTZ19R H3
TG1's (C-test strange) I, II, IV

47.4 (0.3) - rerun of opp. orientation - something wrong ī clone
Hc/Pvu PTZ DH5αF'

Sequencing Region 47.4

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3/15/18

47.4 0.3 Hinc/Pvu (5') - XIII, XIV
FV (0.9 Dde I) - 5
TN (3.0 Eg I) - 3
FV (0.5 HB) - III, IV, VI

m13 -12
m13
m13
PT2

Annealing mix

① primer (universal) 8λ
10x TM 6λ

② Primer (reverse) 14λ
10x TM 10.5λ

13λ per 10ml Acryl mix

50ml in beaker - thin lipped Add 65λ Temed, APS
10ml 2.5mix - 13λ Temed, Aps
10ml 0.5 mix - " "

10mM Tris 40μl
Klenow 4μl
S^{35} 30μl

Template 2μl — spin
Dideoxy 2μl — spin
Mix 2μl — spin

FV .5 III IV
VI 6 0.5

*Loaded
1 FV(5) VI TCAG

2 FV(9) 5
5 TN(3.0) 3
4 7.4(0.3) XIII
3 FV(5) XIV
III
4 IV